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(54) Title: TRANSFERRIN RECEPTOR GENES OF *MORAXELLA*

(57) Abstract

Purified and isolated nucleic acid molecules are provided which encode Tbp2 proteins of *M. catarrhalis* strains M35, 3 and LES1. The nucleic acid sequence may be used to produce recombinant Tbp2 proteins of the strain of *Moraxella* free of other proteins of the *Moraxella* strain for purposes of diagnostics and medical treatment. Furthermore, the nucleic acid molecules may be used in the diagnosis of infection.

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TITLE OF INVENTION

TRANSFERRIN RECEPTOR GENES OF *MORAXELLA*

FIELD OF INVENTION

The present invention relates to the molecular cloning of genes encoding transferrin receptor (TfR) proteins and, in particular, to the cloning of transferrin receptor genes from *Moraxella (Branhamella) catarrhalis*.

BACKGROUND OF THE INVENTION

Moraxella (Branhamella) catarrhalis bacteria are Gram-negative diplococcal pathogens which are carried asymptomatically in the healthy human respiratory tract. In recent years, *M. catarrhalis* has been recognized as an important causative agent of otitis media. In addition, *M. catarrhalis* has been associated with sinusitis, conjunctivitis, and urogenital infections, as well as with a number of inflammatory diseases of the lower respiratory tract in children and adults, including pneumonia, chronic bronchitis, tracheitis, and emphysema (refs. 1 to 8). (Throughout this application, various references are cited in parentheses to describe more fully the state of the art to which this invention pertains. Full bibliographic information for each citation is found at the end of the specification, immediately preceding the claims. The disclosures of these references are hereby incorporated by reference into the present disclosure). Occasionally, *M. catarrhalis* invades to cause septicaemia, arthritis, endocarditis, and meningitis (refs. 9 to 13).

Otitis media is one of the most common illnesses of early childhood and approximately 80% of all children suffer at least one middle ear infection before the age

of three (ref. 14). Chronic otitis media has been associated with auditory and speech impairment in children, and in some cases, has been associated with learning disabilities. Conventional treatments for otitis media include antibiotic administration and surgical procedures, including tonsillectomies, adenoidectomies, and tympanocentesis. In the United States, treatment costs for otitis media are estimated to be between one and two billion dollars per year.

In otitis media cases, *M. catarrhalis* commonly is co-isolated from middle ear fluid along with *Streptococcus pneumoniae* and non-typable *Haemophilus influenzae*, which are believed to be responsible for 50% and 30% of otitis media infections, respectively. *M. catarrhalis* is believed to be responsible for approximately 20% of otitis media infections (ref. 15). Epidemiological reports indicate that the number of cases of otitis media attributable to *M. catarrhalis* is increasing, along with the number of antibiotic-resistant isolates of *M. catarrhalis*. Thus, prior to 1970, no β-lactamase-producing *M. catarrhalis* isolates had been reported, but since the mid-seventies, an increasing number of β-lactamase-expressing isolates have been detected. Recent surveys suggest that 75% of clinical isolates produce β-lactamase (ref. 16, 26).

Iron is an essential nutrient for the growth of many bacteria. Several bacterial species, including *M. catarrhalis*, obtain iron from the host by using transferrin receptor proteins to capture transferrin. A number of bacteria including *Neisseria meningitidis* (ref. 17), *N. gonorrhoeae* (ref. 18), *Haemophilus influenzae* (ref. 19), as well as *M. catarrhalis* (ref. 20), produce outer membrane proteins which specifically bind human transferrin. The expression of these

proteins is regulated by the amount of iron in the environment.

The two transferrin receptor proteins of *M. catarrhalis*, designated transferrin binding protein 1 (Tbp1) and transferrin binding protein 2 (Tbp2), have molecular weights of 115 kDa (Tbp1) and approximately 80 to 90 kDa (Tbp2). Unlike the transferrin receptor proteins of other bacteria which have an affinity for apotransferrin, the *M. catarrhalis* Tbp2 receptors have a preferred affinity for iron-saturated (i.e., ferri-) transferrin (ref. 21).

M. catarrhalis infection may lead to serious disease. It would be advantageous to provide a recombinant source of transferrin binding proteins as antigens in immunogenic preparations including vaccines, carriers for other antigens and immunogens and the generation of diagnostic reagents. The genes encoding transferrin binding proteins and fragments thereof are particularly desirable and useful in the specific identification and diagnosis of *Moraxella* and for immunization against disease caused by *M. catarrhalis* and for the generation of diagnostic reagents.

There had previously been described in published PCT application WO 97/32380, assigned to Connaught Laboratories Limited, the assignee hereof, the cloning, subcloning and sequencing of nucleic acid molecules encoding transferrin receptor proteins Tbp1 and Tbp2 of certain specific strains of *Moraxella catarrhalis*, namely *M. catarrhlais* strains 4223, Q8 and R1, as well as identifying the deduced amino acid sequences of the encoded Tbp1 and Tbp2 proteins.

WO 97/32380 further describes the construction of expression plasmids for the production of recombinant Tbp1 from *M. catarrhalis* strain 4223 and of recombinant

Tbp2 from *M. catarrhalis* strains 4223 and Q8, the recombinant expression of such proteins in *E. coli*, and the extraction and purification of the expressed Tbp1 and Tbp2 proteins.

SUMMARY OF THE INVENTION

The present invention is directed towards the provision of purified and isolated nucleic acid molecules encoding the transferrin receptor protein Tbp2 of additional strains of *Moraxella catarrhalis*, namely strains M35, 3 and LES1. As in the case of WO 97/32380, the respective genes encoding the Tbp1 and Tbp2 proteins are identified as *tbpA* and *tbpB* genes.

The nucleic acid molecules provided herein are useful for the specific detection of strains of *Moraxella* and for diagnosis of infection by *Moraxella*. The purified and isolated nucleic acid molecules provided herein, such as DNA, are also useful for expressing the *tbp* genes by recombinant DNA means for providing, in an economical manner, purified and isolated transferrin receptor proteins as well as subunits, fragments or analogs thereof.

The transferrin receptor, subunits or fragments thereof or analogs thereof, as well as nucleic acid molecules encoding the same and vectors containing such nucleic acid molecules, are useful in immunogenic compositions for vaccinating against diseases caused by *Moraxella*, the diagnosis of infection by *Moraxella* and as tools for the generation of immunological reagents.

Monoclonal antibodies or mono-specific antisera (antibodies) raised against the transferrin receptor protein, produced in accordance with aspects of the present invention, are useful for the diagnosis of infection by *Moraxella*, the specific detection of

Moraxella (in, for example, *in vitro* and *in vivo* assays) and for the treatment of diseases caused by *Moraxella*.

In accordance with one aspect of the present invention, there is provided a purified and isolated nucleic acid molecule encoding transferrin receptor protein Tbp2 of a strain of *Moraxella*, specifically *M. catarrhalis* strain M35, 3 or LES1.

In one preferred embodiment of the invention, the nucleic acid molecule may encode only the Tbp2 protein of the *Moraxella* strain.

The purified and isolated nucleic acid molecule preferably has a DNA sequence selected from the group consisting of (a) a DNA sequence as set out in Figure 2, 4 or 6 (SEQ ID NOS: 1, 3 or 5) or the complementary DNA sequence thereto; (b) a DNA sequence encoding an amino acid sequence as set out in Figure 2, 4 or 6 (SEQ ID NOS: 2, 4 or 6) or the complementary DNA sequence thereto.

In an additional aspect, the present invention includes a vector adapted for transformation of a host, comprising a nucleic acid molecule as provided herein. Such vector may further comprise expression means operatively coupled to the nucleic acid molecule for expression by the host of the Tbp2 protein of the respective strain of *M. catarrhalis*.

The expression means may include a promoter and a nucleic acid portion encoding a leader sequence for secretion from the host of the transferrin receptor protein. The expression means also may include a nucleic acid portion encoding a lipidation signal for expression from the host of a lipidated form of the transferrin receptor protein or the fragment. The host transformed by the expression vector may be selected from, for example, *Escherichia coli*, *Bordetella*,

Bacillus, *Haemophilus*, *Moraxella*, fungi, yeast or baculovirus and Semliki Forest virus expression systems may be used.

In an additional aspect of the invention, there is provided a transformed host containing an expression vector as provided herein. The invention further includes a recombinant Tbp2 protein of the specific strains of *Moraxella catarrhalis* and producible by the transformed host. Such recombinant Tbp2 proteins have a deduced amino acid sequence selected from the group consisting of those shown in Figure 2, 4 or 6 (SEQ ID NO: 2, 4 or 6).

Such recombinant transferrin receptor protein may be provided in substantially pure form according to a further aspect of the invention, which provides a method of forming a substantially pure recombinant Tbp2 protein of *Moraxella catarrhalis* strain M35, 3 or LES1, which comprises growing the transformed host provided herein to express Tbp2 protein as inclusion bodies, purifying the inclusion bodies free from cellular material and soluble proteins, solubilizing Tbp2 protein from the purified inclusion bodies, and purifying the Tbp2 protein free from other solubilized materials. The substantially pure recombinant transferrin receptor protein is generally at least about 70% pure, preferably at least about 90% pure.

In accordance with another aspect of the invention, an immunogenic composition is provided which comprises at least one active component selected from at least one nucleic acid molecule as provided herein and at least one recombinant protein as provided herein, and a pharmaceutically acceptable carrier therefor or vector therefor. The at least one active component produces an immune response when administered to a host.

The immunogenic compositions provided herein may be formulated as vaccines for *in vivo* administration to a host. For such purpose, the compositions may be formulated as a microparticle, capsule, ISCOM (immunostimulatory complex) or liposome preparation. The immunogenic composition may be provided in combination with a targeting molecule for delivery to specific cells of the immune system or to mucosal surfaces. The immunogenic compositions of the invention (including vaccines) may further comprise at least one other immunogenic or immunostimulating material and the immunostimulating material may be at least one adjuvant or at least one cytokine.

Suitable adjuvants for use in the present invention include (but are not limited to) aluminum phosphate, aluminum hydroxide, QS21, Quil A, derivatives and components thereof, ISCOM matrix, calcium phosphate, calcium hydroxide, zinc hydroxide, a glycolipid analog, an octadecyl ester of an amino acid, a muramyl dipeptide, polyphosphazene, ISCOPREP, DC-chol, DDBA and a lipoprotein.

Advantageous combinations of adjuvants are described in copending United States Patent Applications Nos. 08/261,194 filed June 16, 1994 and 08/483,856, filed June 7, 1995, assigned to the assignee hereof and the disclosures of which are incorporated herein by reference thereto (WO 95/34308).

In accordance with another aspect of the invention, there is provided a method for generating an immune response in a host, comprising the step of administering to a susceptible host, such as a human, an effective amount of the immunogenic composition provided herein. The immune response may be a humoral or a cell-mediated immune response and may provide protection against disease caused by *Moraxella*. Hosts in which protection

against disease may be conferred include primates, including humans.

In a further aspect of the invention, there is provided a live vector for delivery of Tbp2 protein to a host, comprising a vector containing the nucleic acid molecule as described above. The vector may be selected from *Salmonella*, BCG, adenovirus, poxvirus, vaccinia and poliovirus.

The nucleic acid molecules provided herein are useful in diagnostic applications. Accordingly, in a further aspect of the invention, there is provided a method of determining the presence, in a sample, of nucleic acid encoding a transferrin receptor protein of a strain of *Moraxella*, comprising the steps of:

(a) contacting the sample with a nucleic acid molecule as provided herein to produce duplexes comprising the nucleic acid molecule and any nucleic acid molecule encoding the transferrin receptor protein of a strain of *Moraxella* present in the sample and specifically hybridizable therewith; and

(b) determining the production of the duplexes.

In addition, the present invention provides a diagnostic kit for determining the presence, in a sample, of nucleic acid encoding a transferrin receptor protein of a strain of *Moraxella*, comprising:

(a) a nucleic acid molecule as provided herein;

(b) means for contacting the nucleic acid molecule with the sample to produce duplexes comprising the nucleic acid molecule and any such nucleic acid present in the sample and hybridizable with the nucleic acid molecule; and

(c) means for determining production of the duplexes.

The invention further includes the use of the nucleic acid molecules and proteins provided herein as

medicines. The invention additionally includes the use of the nucleic acid molecules and proteins provided herein in the manufacture of medicaments for protection against infection by strains of *Moraxella*.

Advantages of the present invention include:

- an isolated and purified nucleic acid molecule encoding a Tbp2 protein of specific strains of *Moraxella catarrhalis*;
- recombinantly-produced Tbp2 proteins; and
- diagnostic kits and immunological reagents for specific identification of *Moraxella*.

BRIEF DESCRIPTION OF DRAWINGS

The present invention will be further understood from the following description with reference to the drawings, in which:

Figure 1 shows a partial restriction map of the *M. catarrhalis* strain M35 *tbpB* gene;

Figure 2 shows the nucleotide sequence of the *tbpB* gene (SEQ ID NO: 1) and deduced amino acid sequence of the Tbp2 protein of *M. catarrhalis* strain M35 (SEQ ID NO: 2);

Figure 3 shows a partial restriction map of the *tbpB* gene for *M. catarrhalis* strain 3;

Figure 4 shows the nucleotide sequence of *tbpB* gene (SEQ ID NO: 3) and the deduced amino acid sequence of the Tbp2 protein of *M. catarrhalis* strain 3 (SEQ ID NO: 4);

Figure 5 shows a partial restriction map of the *tbpB* genes for *M. catarrhalis* strain LES1;

Figure 6 shows the nucleotide sequence of the *tbpB* gene (SEQ ID NO: 5) and deduced amino acid sequence of the Tbp2 *M. catarrhalis* strain LES1 (SEQ ID NO: 6);

Figure 7 shows an alignment of the Tbp2 proteins from strains 4223 (SEQ ID NO: 7), R1 (SEQ ID NO: 8),

M35 (SEQ ID NO: 2), LES1 (SEQ ID NO: 6), Q8 (SEQ ID NO: 9) and 3 (SEQ ID NO: 4). Dots indicate identical residues and spaces have been introduced to maximize the sequence alignment. Underlining indicates those sequences conserved amongst the *M. catarrhalis* Tbp2 proteins and those from *A. pleuropneumoniae*, *H. influenzae*, *N. gonorrhoeae*, *N. meningitidis* and *P. haemolytica* (SEQ ID NOS: 7, 8 and 9 are disclosed in WO 97/32380);

Figure 8 shows the nucleotide and deduced amino acid sequences of the *M. catarrhalis* strain 4223 *tbpA* - *orf3* - *tbpB* gene locus (SEQ ID NO: 10 - entire gene locus; SEQ ID NO: 11 - *tbpA* coding sequence; SEQ ID NO: 12 - deduced amino acid sequence of TbpA; SEQ ID NO: 13 - *orf3* coding sequence; SEQ ID NO: 14 - deduced amino acid sequence of ORF3; SEQ ID NO: 15 - *tbpB* coding sequence; SEQ ID NO: 7 - deduced amino acid sequence of Tbp2);

Figure 9 shows an alignment of the ORF3 proteins from *M. catarrhalis* strains 4223 (SEQ ID NO: 14) and Q8 (SEQ ID NO: 16). Dots indicate identical residues;

Figure 10 shows a restriction map of clone LEM3-24 the construction of which is described in WO 97/32380 (ATCC deposit No. 97,381 deposited December 4, 1995) showing the location of the *orf3* gene in addition to the *tbpA* and *tbpB* genes of *M. catarrhalis* strain 4223 (cf. Figure 2 of WO 96/32380); and

Figure 11 shows a restriction map of clone SLRD-A the construction of which is described in WO 97/32380 (ATCC deposit No. 97,381 deposited December 4, 1995), showing the locations of the *orf3* gene in addition to the *tbpA* and *tbpB* genes of *M. catarrhalis* strain Q8 (cf. Figure 7 of WO 97/32380).

GENERAL DESCRIPTION OF THE INVENTION

Moraxella catarrhalis strains M35, 3 and LES1 may be conveniently used to provide the purified and isolated nucleic acid, which may be in the form of DNA molecules, comprising at least a portion of the nucleic acid coding for a Tbp2 protein of the strain. Strains 4223, LES1 and M35 are all derived from patients with otitis media while strains 3, R1 and Q8 were from sputum or bronchial secretions.

The *tbpB* genes from *M. catarrhalis* M35, 3 and LES1 were cloned and sequenced herein, following generally the procedures described in WO 97/32380. Strain 3 is a clinical isolate provided by Dr. T. Murphy (State University of New York, Buffalo, New York); strain M35 was obtained from Dr. G.D. Campbell (Louisiana State University, Shreveport, Louisiana) and strain LES1 was obtained from Dr. L. Stanfors (University of Tromso, Finland).

Figures 2, 4 and 6 show the nucleotide sequences of the respective *tbpB* genes (SEQ ID NO: 1, 3 or 5) and deduced amino acid sequence of the Tbp2 protein (SEQ ID NO: 2, 4 or 6) of the *M. catarrhalis* strains M35, 3 and LES1, respectively. Regions of homology are evident between the *M. catarrhalis* Tbp2 amino acid sequences determined herein and those previously determined in WO 97/32380, as shown in the comparative alignment of Figure 7 (SEQ ID NOS: 7, 8, 2, 6, 9 and 4) and between the *M. catarrhalis* Tbp2 amino acid sequences. Underlining in Figure 7 indicates those sequences which are conserved among the *M. catarrhalis* Tbp2 proteins and those of *A. pleuropneumoniae*, *H. influenzae*, *N. gonorrhoeae*, *N. meningitidis* and *P. haemolytica*.

Sequence analysis of the nucleotide acid and amino acid sequences of the Tbp2 proteins described herein

and in WO 97/32380 indicated that at least two families could be identified for *M. catarrhalis tbpB* genes, one comprising strains 4223, R1 and M35 and other comprising strains Q8 and 3, with strain LES1 being equally related to both families. Anti-rTbp2 bactericidal antibody activity (Table 1) correlated with the putative gene families identified by sequencing.

Additional sequence analysis of the entire *M. catarrhalis* strains 4223 and Q8 *tbpA - tbpB* locus gene sequence (Figure 8) identified an intergenic open reading frame termed "*orf3*" (SEQ ID NO: 13, SEQ ID NO: 14, ORF3 amino acid sequence), (see also Figures 10 and 11 for location of orf3). The encoded ORF3 proteins from 4223 and Q8 are 98% identical, as seen from the sequence alignment of Figure 9 (SEQ ID NOS: 14, 16).

Cloned *tbpB* genes may be expressed in *E. coli* to produce recombinant Tbp2 proteins free of other *Moraxella* proteins. These recombinant proteins may be purified and used for immunization.

The Tbp2 proteins provided herein are useful as a diagnostic reagent, as an antigen for the generation of anti-transferrin protein binding antibodies, as an antigen for vaccination against the disease caused by species of *Moraxella* and for detecting infection by *Moraxella* and other such bacteria.

The Tbp2 proteins provided herein may also be used as a carrier protein for haptens, polysaccharides or peptides to make conjugate vaccines against antigenic determinants unrelated to transferrin binding proteins. In additional embodiments of the present invention, therefore, the Tbp2 proteins as provided herein may be used as a carrier molecule to prepare chimeric molecules and conjugate vaccines (including glycoconjugates)

against pathogenic bacteria, including encapsulated bacteria. Thus, for example, glycoconjugates of the present invention may be used to confer protection against disease and infection caused by any bacteria having polysaccharide antigens including lipooligosaccharides (LOS) and PRP. Such bacterial pathogens may include, for example, *Haemophilus influenzae, Streptococcus pneumoniae, Escherichia coli, Neisseria meningitidis, Salmonella typhi, Streptococcus mutans, Cryptococcus neoformans, Klebsiella, Staphylococcus aureus* and *Pseudomonas aeruginosa*. Particular antigens which can be conjugated to Tbp2 proteins and methods to achieve such conjugations are described in U.S. Patent Application No. 08/433,522 filed November 23, 1993 (WO 94/12641), assigned to the assignee hereof and the disclosure of which is hereby incorporated by reference thereto.

In another embodiment, the carrier function of the Tbp2 proteins may be used, for example, to induce an immune response against abnormal polysaccharides of tumour cells, or to produce anti-tumour antibodies that can be conjugated to chemotherapeutic or bioactive agents.

The invention extends to transferrin binding proteins from *Moraxella catarrhalis* for use as an active ingredient in a vaccine against disease caused by infection with *Moraxella*. The invention also extends to a pharmaceutical vaccinal composition containing transferrin binding proteins from *Moraxella catarrhalis* and optionally, a pharmaceutically acceptable carrier and/or diluent.

In a further aspect the invention provides the use of transferrin binding proteins for the preparation of a

pharmaceutical vaccinal composition for immunization against disease caused by infection with *Moraxella*.

It is clearly apparent to one skilled in the art, that the various embodiments of the present invention have many applications in the fields of vaccination, diagnosis, treatment of, for example, *Moraxella* infections and the generation of immunological and other diagnostic reagents. A further non-limiting discussion of such uses is further presented below.

1. Vaccine Preparation and Use

Immunogenic compositions, suitable to be used as vaccines, may be prepared from immunogenic transferrin receptor proteins, analogs and fragments thereof encoded by the nucleic acid molecules as well as the nucleic acid molecules disclosed herein. The vaccine elicits an immune response which produces antibodies, including anti-transferrin receptor antibodies and antibodies that are opsonizing or bactericidal. Should the vaccinated subject be challenged by *Moraxella*, the antibodies bind to the transferrin receptor and thereby prevent access of the bacteria to an iron source which is required for viability. Furthermore, opsonizing or bactericidal anti-transferrin receptor antibodies may also provide protection by alternative mechanisms.

Immunogenic compositions, including vaccines, may be prepared as injectables, as liquid solutions or emulsions. The transferrin receptor proteins, analogs and fragments thereof and encoding nucleic acid molecules may be mixed with pharmaceutically acceptable excipients which are compatible with the transferrin receptor proteins, fragments, analogs or nucleic acid molecules. Such excipients may include water, saline, dextrose, glycerol, ethanol, and combinations thereof. The immunogenic compositions and vaccines may further

contain auxiliary substances, such as wetting or emulsifying agents, pH buffering agents, or adjuvants, to enhance the effectiveness of the vaccines. Immunogenic compositions and vaccines may be administered parenterally, by injection subcutaneously, intradermally or intramuscularly. Alternatively, the immunogenic compositions provided according to the present invention, may be formulated and delivered in a manner to evoke an immune response at mucosal surfaces. Thus, the immunogenic composition may be administered to mucosal surfaces by, for example, the nasal or oral (intragastric) routes. The immunogenic composition may be provided in combination with a targeting molecule for delivery to specific cells of the immune system or to mucosal surfaces. Some such targeting molecules include vitamin B12 and fragments of bacterial toxins, as described in WO 92/17167 (Biotech Australia Pty. Ltd.), and monoclonal antibodies, as described in U.S. Patent No. 5,194,254 (Barber et al). Alternatively, other modes of administration, including suppositories and oral formulations, may be desirable. For suppositories, binders and carriers may include, for example, polyalkalene glycols or triglycerides. Oral formulations may include normally employed incipients such as, for example, pharmaceutical grades of saccharine, cellulose and magnesium carbonate. These compositions may take the form of solutions, suspensions, tablets, pills, capsules, sustained release formulations or powders and contain about 1 to 95% of the transferrin receptor proteins, fragments, analogs and/or nucleic acid molecules.

The vaccines are administered in a manner compatible with the dosage formulation, and in such amount as will be therapeutically effective, protective and immunogenic. The quantity to be administered

depends on the subject to be treated, including, for example, the capacity of the individual's immune system to synthesize antibodies, and, if needed, to produce a cell-mediated immune response. Precise amounts of active ingredient required to be administered depend on the judgment of the practitioner. However, suitable dosage ranges are readily determinable by one skilled in the art and may be of the order of micrograms of the transferrin receptor proteins, analogs and fragments thereof and/or nucleic acid molecules. Suitable regimes for initial administration and booster doses are also variable, but may include an initial administration followed by subsequent administrations. The dosage of the vaccine may also depend on the route of administration and will vary according to the size of the host.

The nucleic acid molecules encoding the transferrin receptor of *Moraxella* may be used directly for immunization by administration of the DNA directly, for example, by injection for genetic immunization or by constructing a live vector, such as *Salmonella*, BCG, adenovirus, poxvirus, vaccinia or poliovirus containing the nucleic acid molecules. A discussion of some live vectors that have been used to carry heterologous antigens to the immune system is contained in, for example, O'Hagan (ref. 22). Processes for the direct injection of DNA into test subjects for genetic immunization are described in, for example, Ulmer et al. (ref. 23).

Immunogenicity can be significantly improved if the antigens are co-administered with adjuvants, commonly used as an 0.05 to 1.0 percent solution in phosphate - buffered saline. Adjuvants enhance the immunogenicity of an antigen but are not necessarily immunogenic themselves. Adjuvants may act by retaining the antigen

locally near the site of administration to produce a depot effect facilitating a slow, sustained release of antigen to cells of the immune system. Adjuvants can also attract cells of the immune system to an antigen depot and stimulate such cells to elicit immune responses.

Immunostimulatory agents or adjuvants have been used for many years to improve the host immune responses to, for example, vaccines. Intrinsic adjuvants, such as lipopolysaccharides, normally are the components of killed or attenuated bacteria used as vaccines. Extrinsic adjuvants are immunomodulators which are typically non-covalently linked to antigens and are formulated to enhance the host immune responses. Thus, adjuvants have been identified that enhance the immune response to antigens delivered parenterally. Some of these adjuvants are toxic, however, and can cause undesirable side-effects, making them unsuitable for use in humans and many animals. Indeed, only aluminum hydroxide and aluminum phosphate (collectively commonly referred to as alum) are routinely used as adjuvants in human and veterinary vaccines. The efficacy of alum in increasing antibody responses to diphtheria and tetanus toxoids is well established and an HBsAg vaccine has been adjuvanted with alum. While the usefulness of alum is well established for some applications, it has limitations. For example, alum is ineffective for influenza vaccination and inconsistently elicits a cell mediated immune response. The antibodies elicited by alum-adjuvanted antigens are mainly of the IgG1 isotype in the mouse, which may not be optimal for protection by some vaccinal agents.

A wide range of extrinsic adjuvants can provoke potent immune responses to antigens. These include saponins complexed to membrane protein antigens (immune

stimulating complexes), pluronic polymers with mineral oil, killed mycobacteria and mineral oil, Freund's complete adjuvant, bacterial products, such as muramyl dipeptide (MDP) and lipopolysaccharide (LPS), as well as lipid A, and liposomes.

To efficiently induce humoral immune responses (HIR) and cell-mediated immunity (CMI), immunogens are often emulsified in adjuvants. Many adjuvants are toxic, inducing granulomas, acute and chronic inflammations (Freund's complete adjuvant, FCA), cytolysis (saponins and pluronic polymers) and pyrogenicity, arthritis and anterior uveitis (LPS and MDP). Although FCA is an excellent adjuvant and widely used in research, it is not licensed for use in human or veterinary vaccines because of its toxicity.

Desirable characteristics of ideal adjuvants include:

(1) lack of toxicity;

(2) ability to stimulate a long-lasting immune response;

(3) simplicity of manufacture and stability in long-term storage;

(4) ability to elicit both CMI and HIR to antigens administered by various routes, if required;

(5) synergy with other adjuvants;

(6) capability of selectively interacting with populations of antigen presenting cells (APC);

(7) ability to specifically elicit appropriate T_H1 or T_H2 cell-specific immune responses; and

(8) ability to selectively increase appropriate antibody isotype levels (for example, IgA) against antigens.

U.S. Patent No. 4,855,283 granted to Lockhoff et al on August 8, 1989, which is incorporated herein by reference thereto, teaches glycolipid analogues

including N-glycosylamides, N-glycosylureas and N-glycosylcarbamates, each of which is substituted in the sugar residue by an amino acid, as immuno-modulators or adjuvants. Thus, Lockhoff et al. 1991 (ref. 24) reported that N-glycolipid analogs displaying structural similarities to the naturally-occurring glycolipids, such as glycophospholipids and glycoglycerolipids, are capable of eliciting strong immune responses in both herpes simplex virus vaccine and pseudorabies virus vaccine. Some glycolipids have been synthesized from long chain-alkylamines and fatty acids that are linked directly with the sugars through the anomeric carbon atom, to mimic the functions of the naturally occurring lipid residues.

U.S. Patent No. 4,258,029 granted to Moloney, assigned to the assignee hereof and incorporated herein by reference thereto, teaches that octadecyl tyrosine hydrochloride (OTH) functions as an adjuvant when complexed with tetanus toxoid and formalin inactivated type I, II and III poliomyelitis virus vaccine. Also, Nixon-George et al. 1990, (ref. 25) reported that octadecyl esters of aromatic amino acids complexed with a recombinant hepatitis B surface antigen, enhanced the host immune responses against hepatitis B virus.

2. Immunoassays

The transferrin receptor proteins, analogs and/or fragments thereof of the present invention are useful as immunogens, as antigens in immunoassays including enzyme-linked immunosorbent assays (ELISA), RIAs and other non-enzyme linked antibody binding assays or procedures known in the art for the detection of anti-*Moraxella*, transferrin receptor protein antibodies. In ELISA assays, the transferrin receptor protein, analogs and/or fragments corresponding to portions of TfR protein, are immobilized onto a selected surface, for

example, a surface capable of binding proteins or peptides such as the wells of a polystyrene microtiter plate. After washing to remove incompletely adsorbed transferrin receptor, analogs and/or fragments, a non-specific protein such as a solution of bovine serum albumin (BSA) or casein that is known to be antigenically neutral with regard to the test sample may be bound to the selected surface. This allows for blocking of nonspecific adsorption sites on the immobilizing surface and thus reduces the background caused by non-specific bindings of antisera onto the surface.

The immobilizing surface is then contacted with a sample, such as clinical or biological materials, to be tested in a manner conducive to immune complex (antigen/antibody) formation. This procedure may include diluting the sample with diluents, such as BSA, bovine gamma globulin (BGG) and/or phosphate buffered saline (PBS)/Tween. The sample is then allowed to incubate for from about 2 to 4 hours, at temperatures such as of the order of about 25° to 37°C. Following incubation, the sample-contacted surface is washed to remove non-immunocomplexed material. The washing procedure may include washing with a solution such as PBS/Tween or a borate buffer.

Following formation of specific immunocomplexes between the test sample and the bound transferrin receptor protein, analogs and/or fragments and subsequent washing, the occurrence, and even amount, of immunocomplex formation may be determined by subjecting the immunocomplex to a second antibody having specificity for the first antibody. If the test sample is of human origin, the second antibody is an antibody having specificity for human immunoglobulins and in general IgG. To provide detecting means, the second

antibody may have an associated activity such as an enzymatic activity that will generate, for example, a color development upon incubating with an appropriate chromogenic substrate. Quantification may then achieved by measuring the degree of color generation using, for example, a spectrophotometer.

3. Use of Sequences as Hybridization Probes

The nucleotide sequences of the present invention, comprising the sequence of the transferrin receptor gene, now allow for the identification and cloning of the transferrin receptor genes from any species of *Moraxella*.

The nucleotide sequences comprising the sequence of the transferrin receptor genes of the present invention are useful for their ability to selectively form duplex molecules with complementary stretches of other TfR genes. Depending on the application, a variety of hybridization conditions may be employed to achieve varying degrees of selectivity of the probe toward the other TfR genes. For a high degree of selectivity, relatively stringent conditions are used to form the duplexes, such as low salt and/or high temperature conditions, such as provided by 0.02 M to 0.15 M NaCl at temperatures of between about 50°C to 70°C. For some applications, less stringent hybridization conditions are required such as 0.15 M to 0.9 M salt, at temperatures ranging from between about 20°C to 55°C. Hybridization conditions can also be rendered more stringent by the addition of increasing amounts of formamide, to destabilize the hybrid duplex. Thus, particular hybridization conditions can be readily manipulated, and will generally be a method of choice depending on the desired results. In general, convenient hybridization temperatures in the presence of 50%

formamide are: 42°C for a probe which is 95 to 100% homologous to the target fragment, 37°C for 90 to 95% homology and 32°C for 85 to 90% homology.

In a clinical diagnostic embodiment, the nucleic acid sequences of the TfR genes of the present invention may be used in combination with an appropriate means, such as a label, for determining hybridization. A wide variety of appropriate indicator means are known in the art, including radioactive, enzymatic or other ligands, such as avidin/biotin and digoxigenin-labelling, which are capable of providing a detectable signal. In some diagnostic embodiments, an enzyme tag such as urease, alkaline phosphatase or peroxidase, instead of a radioactive tag may be used. In the case of enzyme tags, colorimetric indicator substrates are known which can be employed to provide a means visible to the human eye or spectrophotometrically, to identify specific hybridization with samples containing TfR gene sequences.

The nucleic acid sequences of TfR genes of the present invention are useful as hybridization probes in solution hybridizations and in embodiments employing solid-phase procedures. In embodiments involving solid-phase procedures, the test DNA (or RNA) from samples, such as clinical samples, including exudates, body fluids (e. g., serum, amniotic fluid, middle ear effusion, sputum, bronchoalveolar lavage fluid) or even tissues, is adsorbed or otherwise affixed to a selected matrix or surface. The fixed, single-stranded nucleic acid is then subjected to specific hybridization with selected probes comprising the nucleic acid sequences of the TfR genes or fragments thereof of the present invention under desired conditions. The selected conditions will depend on the particular circumstances

based on the particular criteria required depending on, for example, the G+C contents, type of target nucleic acid, source of nucleic acid, size of hybridization probe etc. Following washing of the hybridization surface so as to remove non-specifically bound probe molecules, specific hybridization is detected, or even quantified, by means of the label. It is preferred to select nucleic acid sequence portions which are conserved among species of *Moraxella*. The selected probe may be at least 18bp and may be in the range of about 30 to 90 bp.

4. Expression of the Transferrin Receptor Genes

Plasmid vectors containing replicon and control sequences which are derived from species compatible with the host cell may be used for the expression of the transferrin receptor genes in expression systems. The vector ordinarily carries a replication site, as well as marking sequences which are capable of providing phenotypic selection in transformed cells. For example, *E. coli* may be transformed using pBR322 which contains genes for ampicillin and tetracycline resistance and thus provides easy means for identifying transformed cells. The pBR322 plasmid, or other microbial plasmid or phage, must also contain, or be modified to contain, promoters which can be used by the host cell for expression of its own proteins.

In addition, phage vectors containing replicon and control sequences that are compatible with the host can be used as a transforming vector in connection with these hosts. For example, the phage in lambda GEM™-11 may be utilized in making recombinant phage vectors which can be used to transform host cells, such as *E. coli* LE392.

Promoters commonly used in recombinant DNA construction include the β-lactamase (penicillinase) and lactose promoter systems and other microbial promoters, such as the T7 promoter system as described in U.S. Patent No. 4,952,496. Details concerning the nucleotide sequences of promoters are known, enabling a skilled worker to ligate them functionally with genes. The particular promoter used will generally be a matter of choice depending upon the desired results. Hosts that are appropriate for expression of the transferrin receptor genes, fragments, analogs or variants thereof, may include *E. coli*, *Bacillus* species, *Haemophilus*, fungi, yeast, *Moraxella*, *Bordetella*, or the baculovirus expression system may be used.

In accordance with this invention, it is preferred to make the transferrin receptor protein, fragment or analog thereof, by recombinant methods, particularly since the naturally occurring TfR protein as purified from a culture of a species of *Moraxella* may include trace amounts of toxic materials or other contaminants. This problem can be avoided by using recombinantly produced TfR protein in heterologous systems which can be isolated from the host in a manner to minimize contaminants in the purified material. Particularly desirable hosts for expression in this regard include Gram positive bacteria which do not have LPS and are, therefore, endotoxin free. Such hosts include species of *Bacillus* and may be particularly useful for the production of non-pyrogenic transferrin receptor, fragments or analogs thereof.

EXAMPLES

The above disclosure generally describes the present invention. A more complete understanding can be obtained by reference to the following specific

Examples. These Examples are described solely for purposes of illustration and are not intended to limit the scope of the invention. Changes in form and substitution of equivalents are contemplated as circumstances may suggest or render expedient. Although specific terms have been employed herein, such terms are intended in a descriptive sense and not for purposes of limitations.

Methods of molecular genetics, protein biochemistry and immunology used but not explicitly described in this disclosure and these Examples are amply reported in the scientific literature and are well within the ability of those skilled in the art.

Example 1

This Example illustrates the preparation of chromosomal DNA from *M. catarrhalis* strain M35, following the procedure described in WO 97/32380 for strains 4223 and Q8 (Example 2).

M. catarrhalis isolate M35 was inoculated into 100 ml of BHI broth, and incubated for 18 hr at 37°C with shaking. The cells were harvested by centrifugation at 10,000 x g for 20 min. The pellet was used for extraction of *M. catarrhalis* M35 chromosomal DNA.

The cell pellet was resuspended in 20 ml of 10 mM Tris-HCl (pH 7.5)-1.0 mM EDTA (TE). Pronase and SDS were added to final concentrations of 500 µg/ml and 1.0%, respectively, and the suspension was incubated at 37°C for 2 hr. After several sequential extractions with phenol, phenol:chloroform (1:1), and chloroform:isoamyl alcohol (24:1), the aqueous extract was dialysed, at 4°C, against 1.0 M NaCl for 4 hr, and against TE (pH 7.5) for a further 48 hr with three buffer changes. Two volumes of ethanol were added to the dialysate, and the DNA was spooled onto a glass rod.

The DNA was allowed to air-dry, and was dissolved in 3.0 ml of water. Concentration was estimated, by UV spectrophotometry, to be about 290 µg/ml. This procedure was repeated for the preparation of chromosomal DNA from *M. catarrhalis* strain 3 and LES1.

Example 2

This Example illustrates the construction of a *M. catarrhalis* strain M35 chromosomal library in EMBL3.

A series of *Sau*3A restriction digests of chromosomal DNA from *M. catarrhalis* M35, prepared as described in Example 1, in final volumes of 10 µL each, were carried out in order to optimize the conditions necessary to generate maximal amounts of restriction fragments within a 15 to 23 kb size range. Using the optimized digestion conditions, a large-scale digestion was set up in a 100 µL volume, containing the following: 50 µL of chromosomal DNA (290 µg/ml), 33 µL water, 10 µL 10X *Sau*3A buffer (New England Biolabs), 1.0 µL BSA (10 mg/ml, New England Biolabs), and 6.3 µL *Sau*3A (0.04 U/µL). Following a 15 min. incubation at 37°C, the digestion was terminated by the addition of 10 µL of 100 mM Tris-HCl (pH 8.0)-10 mM EDTA-0.1% bromophenol blue-50% glycerol (loading buffer). Digested DNA was electrophoresed through a 0.5% agarose gel in 40 mM Tris acetate-2 mM $Na_2EDTA.2H_2O$ (pH8.5)(TAE buffer) at 50 V for 6 hr. The region containing restriction fragments within a 15 to 23 kb molecular size range was excised from the gel, and placed into dialysis tubing containing 3.0 ml of TAE buffer. DNA was electroeluted from the gel fragment by applying a field strength of 1.0 V/cm for 18 hr. Electroeluted DNA was extracted once each with phenol and phenol:chloroform (1:1), and

precipitated with ethanol. The dried DNA was dissolved in 5.0 µL water.

Size-fractionated chromosomal DNA was ligated with *Bam*HI-digested EMBL3 arms (Promega), using T4 DNA ligase in a final volume of 9 µL. The entire ligation mixture was packaged into lambda phage using a commercial packaging kit (Amersham), following manufacturer's instructions.

The packaged DNA library was amplified on solid media. 0.1 ml aliquots of *Escherichia coli* strain NM539 in 10 mM $MgSO_4$ (OD_{260} = 0.5) were incubated at 37°C for 15 min. with 15 to 25 µL of the packaged DNA library. Samples were mixed with 3 ml of 0.6% agarose containing 1.0% BBL trypticase peptone-0.5% NaCl (BBL top agarose), and mixtures were plated onto 1.5% agar plates containing 1.0% BBL trypticase peptone-0.5% NaCl, and incubated at 37°C for 18 hr. 3 ml quantities of 50 mM Tris-HCl (pH 7.5)-8 mM magnesium sulfate heptahydrate-100 mM NaCl-0.01% (w/v) gelatin (SM buffer) were added to each plate, and plates were left at 4°C for 7 hr. SM buffer containing phage was collected from the plates, pooled together, and stored in a screwcap tube at 4°C, with chloroform.

Example 3

This Example illustrates screening of the *M. catarrhalis* strain M35 library.

The EMBL3/M35 library, prepared as described in Example 2, was plated onto LE392 cells on YT plates using 0.7% top agar in YT as overlay. Plaques were lifted onto nitrocellulose filters and the filters were probed with oligonucleotide probes labelled with $^{32}P\alpha$-dCTP (Random Primed DNA labeling kit, Boehringer Mannheim). The pre-hybridization was performed in sodium chloride/sodium citrate (SSC) buffer (ref. 27) at

37°C for 1 hour and the hybridization was performed at 42°C overnight. The probes were based upon an internal sequence of 4223 *tbpA*:

I R D L T R Y D P G

(SEQ ID No. 17)

4236-RD 5' ATTCGAGACTTAACACGCTATGACCCTGGC 3'

(Seq ID No 18)

4237-RD 5' ATTCGTGATTTAACTCGCTATGACCCTGGT 3'

(Seq ID No 19).

Putative plaques were re-plated and submitted to second and third rounds of screening using the same procedures.

Phage clone M35-2.3 was found to contain a 13 kb insert of the M35 *tfr* genes. The *tbpB* gene was localized to a 7.5 kb Nhel - Sal I fragment by restriction enzyme and Southern blot analyses and was subcloned into pBR328 for sequence analysis, generating plasmid pLEM40.

A partial restriction map of the M35 *tbpB* gene is shown in Figure 1. The nucleotide and deduced amino acid sequences of the M35 *tbpB* gene are shown in Figure 2. The M35 *tbpB* gene encodes a 706 amino acid protein of molecular weight 76.5 kDa. When the M35 TbpB sequence was aligned with the 4223 TbpB protein (Figure 7), it was found to be 86% identical and 90% similar.

Example 4

This Example illustrates the PCR amplification of the *tbpB* genes from *M. catarrhalis* strains 3 and LES1, following the procedure described in WO 97/32380 for *M. catarrhalis* strain R1.

Oligonucleotide primers were based upon the following sequences, which are found in the intergenic regions surrounding *M. catarrhalis* strain 4223 *tbpB*:

5' GATGGGATAAGCACGCCCTACTT 3' (SEQ ID NO: 20)
sense primer (4940)

5' CCCATCAGCCAAACAAACATTGTGT 3' (SEQ ID NO: 21)
antisense primer (4967)

PCR amplification was performed in buffer containing 100 mM Tris-HCI (pH 8.9), 25 mM KCI, 5 mM $(NH_4)_2SO_4$ and 2 mM $MgSO_4$. Each 100 µl reaction mixture contained 10 ng of chromosomal DNA from strains 3 and LES1, prepared following the procedure of Example 1, 1 µg each primer, 2.5 U Pwo DNA polymerase (Boehringer Mannheim) and 0.2 mM dNTPs (Perkin Elmer, Foster City, California). The cycling conditions were 25 cycles of 95°C for 30 sec, 45°C for 1.0 min and 72°C for 2.0 min, followed by a 10 min elongation at 72°C. Specific 2.4 kb fragments were amplified and DNA was purified for direct sequencing by agarose gel extraction, using a Geneclean kit (Bio 101 Inc., Vista, California). Plasmid DNA for sequencing was prepared using a Qiagen Plasmid Midi kit (Qiagen, Chatsworth, California). DNA samples were sequenced using an ABI model 373A DNA sequencer using dye terminator chemistry. Oligonucleotide primers of 17 to 25 bases in length were used to sequence both strands of the genes.

Partial restriction maps of the *M. catarrhalis* strains 3 and LES1 *tbpB* genes are shown in Figures 3 and 5 respectively. The nucleotide and deduced amino acid sequences of the strain 3 and LES1 *tbpB* genes are shown in Figures 4 and 6, respectively. The strain 3 *tbpB* gene encodes a 712 amino acid protein of molecular weight 76.9 kDa, which is more closely related to the strain Q8 Tbp2 protein than to the 4223 Tbp2 protein (Figure 7). The Q8 and strain 3 Tbp2 proteins are 71% identical and 79% similar, whereas the 4223 and strain 3 Tbp2 proteins are 51% identical and 64% similar. The strain LES1 *tbpB* gene encodes a 713 amino acid protein

of molecular weight 76.8 kDa which is 63% identical to both the 4223 and Q8 Tbp2 proteins.

From the sequence analysis presented herein and in further consideration of the sequences presented in WO 98/32380, there appear to be at least two gene families which can be identified for *M. catarrhalis tbpB*, one comprising strains 4223, R1 and M35 and the other comprising strains Q8 and 3, with strain LES1 being equally related to both families. This novel finding is similar to that of the *N. meningitidis tbpB* genes which can be divided into two sub-groups (ref. 28). There is limited sequence homology among the amino acid sequences of the *M. catarrhalis* Tbp2 proteins previously identified in WO 98/32380 and in this application and those from other organisms, such as *Actinobacillus pleuropneumoniae*, *H. influenzae*, *N. gonorrhoeae*, *N. meningitidis* and *P. haemolytical* (ref. 29). The homology is scattered in small peptide motifs throughout the sequence and is illustrated by underlining in Figure 7. The conserved LEGGFYG (SEQ ID NO: 22) epitope was present, as found in Tbp2 for other *M. catarrhalis* strains as well as the *H. influenzae* and *N. meningitidis* Tbp2 proteins.

Example 5

This Example illustrates the bactericidal antibody activity of guinea pig anti-4223 rTbp2 and anti-Q8 rTbp2 antibodies, prepared as described in WO 97/32380 (Example 14), and confirmation of the gene families of *tbpB* genes.

The bactericidal antibody assay was performed as described by Yang et al. (ref. 30). Briefly, several *M. catarrhalis* strains were grown to an OD_{578} of 0.5 in BHI medium containing 25 mM EDDA. The bacteria were diluted so that the pre-bleed control plates contained

100 to 300 cfu. Guinea pig anti-rTbp2 antisera and pre-bleed controls, prepared as described in Example 14 of WO 97/32380, were heated to 56°C for 30 min to inactivate endogenous complement and were diluted 1:64 with veronal buffer containing 0.1% BSA (VBS). Guinea pig complement was diluted 1:10 in VBS. Twenty-five µl each of diluted antiserum, bacteria and complement were added to duplicate wells of a 96 well microtiter plate. The plates were incubated at 37°C for 60 min, gently shaking at 70 rpm on a rotary platform. Fifty µl of each reaction mixture were plated onto Mueller Hinton agar plates which were incubated at 37°C for 24 h, then room temperature for 24 h, before the bacteria were counted. Antisera were determined to be bactericidial if $\geq$50% of bacteria were killed compared with negative controls. Each assay was repeated at least twice in duplicate. The assay was performed using both the anti-Tbp2 antisera from both 4223 and Q8 strains against a number of different strains of *Moraxella catarrhalis*. The strains tested are identified and the results obtained are shown in Table 1.

The anti-rTbp2 bactericidal antibody activity shown in Table 1 corelates with the putative gene families identified by sequencing, as described in Example 4. Anti-4223 rTbp2 antibody kills those strains within its own family, i.e. 4223, R1 and M35, while anti-Q8 rTbp2 antibody kills those strains within its family, i.e. Q8, 3 and LES1. The anti-4223 rTbp2 antibody also killed strains VH-9, H-04 and ATCC 25240 indicating that the latter strains may be part of the 4223 family. Strain H-04 was also killed by anti-Q8 rTbp2 antibody.

Example 6

This Example illustrates the sequence analysis of the open reading frame (ORF) within the intergenic region between *M. catarrhalis tbpA* and *tbpB*.

The intergenic region was sequenced for strains 4223 and Q8 and a single open reading frame was identified. This *orf*, identified as *orf3*, was located about 1 kb downstream of *tbpA* and about 273 bp upstream of *tbpB* in each genome (Figure 10 - strain 4223; Figure 11 - strain Q8). The nucleotide and deduced amino acid sequences of the entire 4223 *tbpA - orf3 - tbpB* gene loci are shown in Figure 8. The encoded 4223 and Q8 ORF3 proteins are 98% identical, 512 amino acid proteins, of molecular weight 58.1 kDa and 57.9 kDa, respectively. The alignment of the ORF3 protein sequences is shown in Figure 9.

SUMMARY OF THE DISCLOSURE

In summary of this disclosure, the present invention provides purified and isolated DNA molecules containing transferrin receptor genes of specific strains of *Moraxella catarrhalis*, the sequences of these transferrin receptor genes, and the derived amino acid sequences of the Tbp2 proteins encoded thereby. The genes and DNA sequences are useful for diagnosis, immunization, and the generation of diagnostic and immunological reagents. Immunogenic compositions, including vaccines, based upon expressed recombinant Tbp1 and/or Tbp2, portions thereof, or analogs thereof, can be prepared for prevention of diseases caused by *Moraxella*. Modifications are possible within the scope of this invention.

TABLE I

Bactericidal antibody activity of guinea pig anti-rTbpB antisera

M. catarrhalis strain	Bactericidal Antibody Activity*	
	Anti-4223 rTbp2	Anti-Q8 rTbp2
4223	++	-
M35	++	-
R1	++	-
LES1	-	+
Q8	-	++
3	-	±
VH-9	++	-
H-04	++	++
ATCC 25240	**	-

* killing by antiserum diluted 1:64 compared to negative controls: - indicates 0 to 25% killing; ± indicates 26 to 49%; + indicates 50 to 75%; ++ indicates 76 to 100% killing.

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CLAIMS

What we claim is:

1. A purified and isolated nucleic acid molecule encoding a Tbp2 protein of a strain of *Moraxella* which strain is selected from the group consisting of *Moraxella catarrhalis* M35, 3 and LES1.

2. The purified and isolated nucleic acid molecule of claim 1, having a DNA sequence selected from the group consisting of:

(a) a DNA sequence as set out in Figure 2, 4 or 6 (SEQ ID NOS: 1, 3 or 5) or the complementary DNA sequence thereto; or

(b) a DNA sequence encoding an amino acid sequence as set out in Figure 2, 4 or 6 (SEQ ID NOS: 2, 4 or 6) or the complementary DNA sequence thereto.

3. A vector adapted for transformation of a host comprising the nucleic acid molecule of claim 1.

4. The vector of claim 3 further comprising expression means operatively coupled to the nucleic acid molecule for expression by the host of said Tbp2 protein of a *Moraxella catarrhalis* strain M35, 3 or LES1.

5. A transformed host containing an expression vector as claimed in claim 4.

6. A method of forming a substantially pure recombinant Tbp2 protein of a *Moraxella catarrhalis* strain M35, 3 or LES1 which comprises:

growing the transformed host of claim 5 to express Tbp2 protein as inclusion bodies,

purifying the inclusion bodies free from cellular material and soluble proteins,

solubilizing Tbp2 protein from the purified inclusion bodies, and

purifying the Tbp2 protein free from other solubilized materials.

7. A recombinant Tbp2 protein of *Moraxella catarrhalis* strain M35, 3 or LES1 producible by the transformed host of claim 5, having a deduced amino acid sequence selected from the group consisting of those shown in Figure 2, 4 or 6 (SEQ ID NO: 2, 4 or 6).

8. An immunogenic composition, comprising at least one active component selected from the group consisting of:

(A) a purified and isolated nucleic acid molecule as claimed in claim 1; or

(B) a recombinant Tbp2 protein as claimed in claim 7;

and a pharmaceutically acceptable carrier therefor, said at least one active component producing an immune response when administered to a host.

9. A method for generating an immune response in a host, comprising administering to the host an immunoeffective amount of the immunogenic composition of claim 8.

10. A method of determining the presence, in a sample, of nucleic acid encoding a transferrin receptor protein of a strain of *Moraxella*, comprising the steps of:

(a) contacting the sample with the nucleic acid molecule of claim 1 to produce duplexes comprising the nucleic acid molecule and any said nucleic acid molecule encoding the transferrin receptor protein of a strain of *Moraxella* present in the sample and specifically hybridizable therewith; and

(b) determining production of the duplexes.

11. A diagnostic kit for determining the presence, in a sample, of nucleic acid encoding a transferrin receptor protein of a strain of *Moraxella*, comprising:

(a) the nucleic acid molecule of claim 1;

(b) means for contacting the nucleic acid molecule with the sample to produce duplexes comprising the nucleic acid molecule and any said nucleic acid present in the sample and hybridizable with the nucleic acid molecule; and

(c) means for determining production of the duplexes.

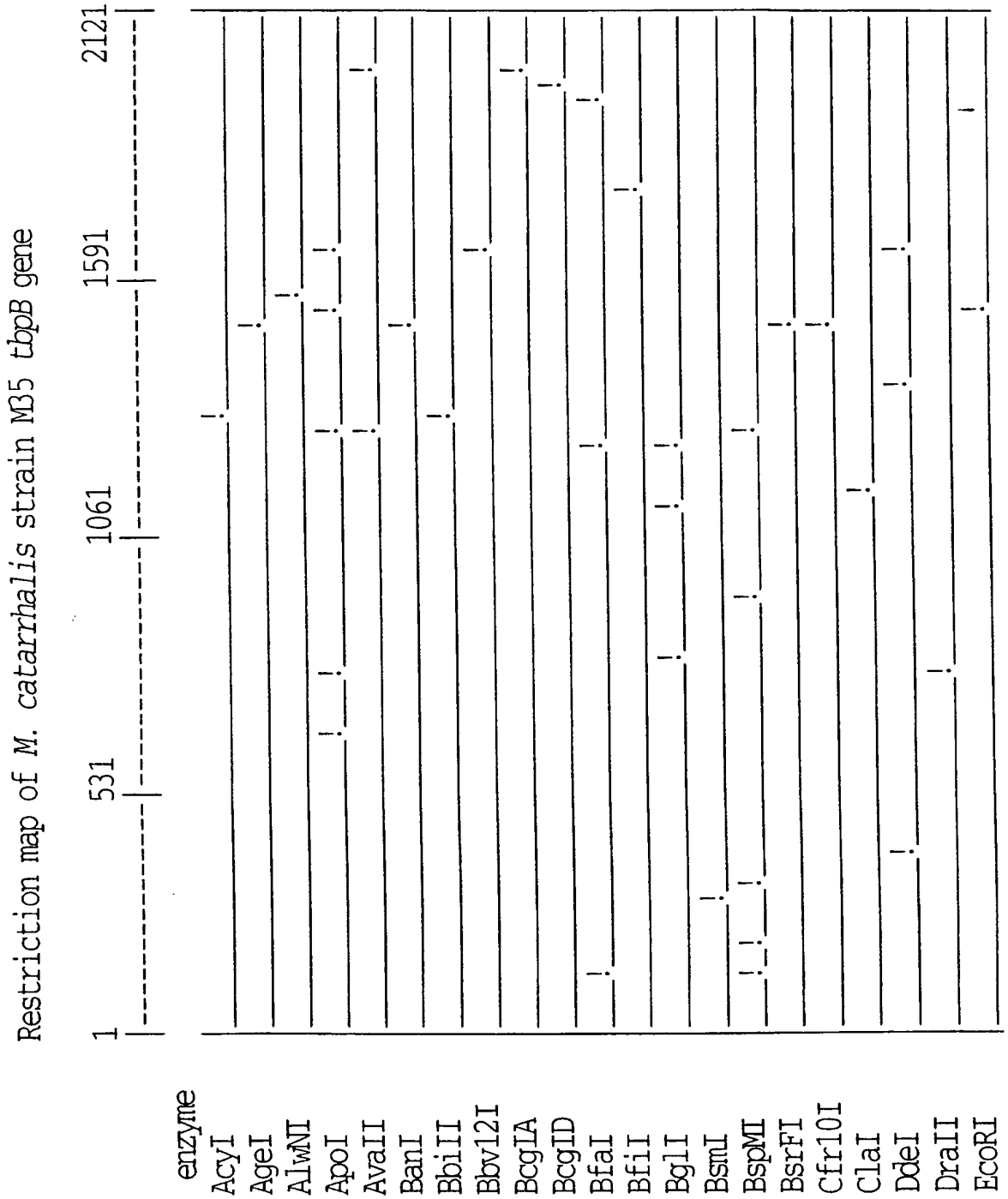
12. A nucleic acid molecule of claim 1 when used as a medicine.

13. A recombinant transferrin receptor protein of claim 7 when used as a medicine.

14. The use of a nucleic acid molecule of claim 1 in the manufacture of a medicament for protection against infection by a strain of *Moraxella*.

15. The use of a recombinant transferrin receptor protein of claim 7 in the manufacture of a medicament for protection against infection by a strain of *Moraxella*.

FIG.1A


Restriction map of M. catarrhalis strain M35 tbpB gene
1
531
1061
1591
2121
enzyme
AcyI
AgeI
AlwNI
ApoI
AvaII
BanI
BbiII
Bbv12I
BcgIA
BcgID
BfaI
BfiI
BglI
BsmI
BspMI
BsrFI
Cfr10I
ClaI
DdeI
DraII
EcoRI

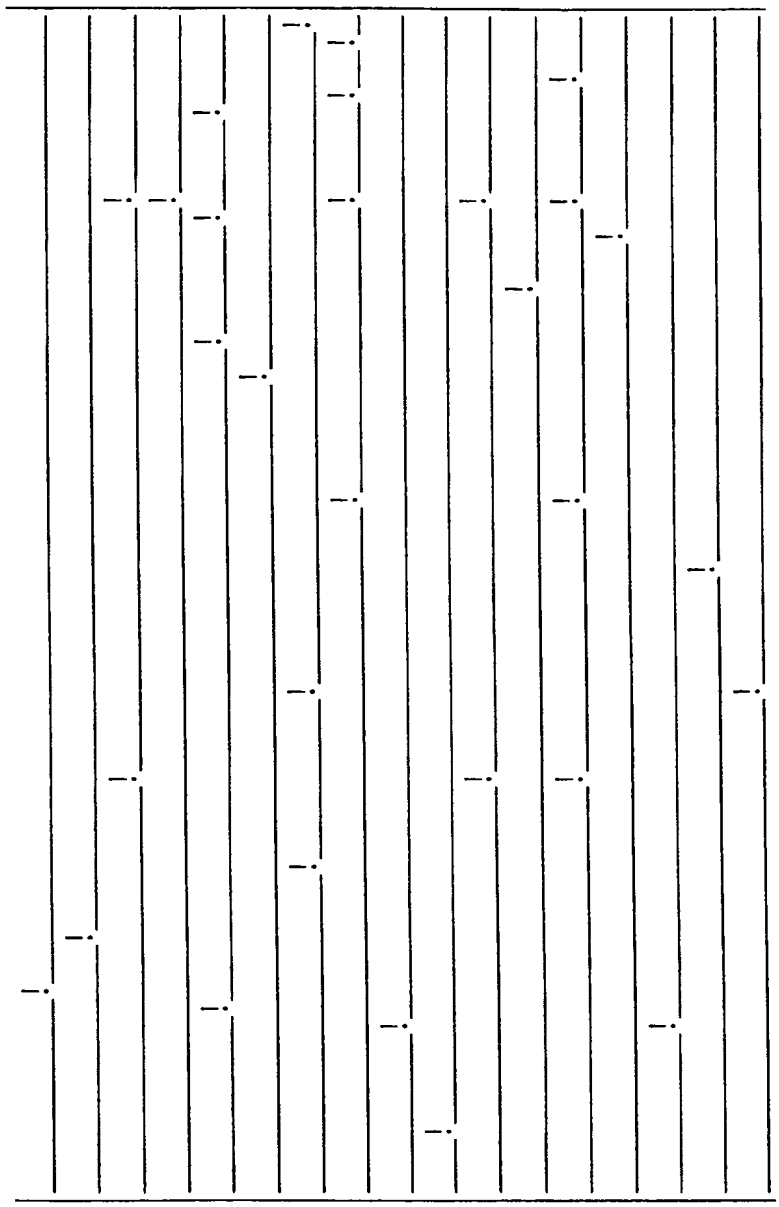

EcoRV
FokI
HaeIII
HindIII
HinfI
HpaII
MboII
MnlI
NcoI
NheI
PalI
SacI
Sau96I
SspI
StyI
TaqI
XmnI

FIG.1B

FIG.2A

M. catarrhalis strain M35 *tbpB* sequence

```
 MET  LYS  HIS  ILE  PRO  LEU  THR  THR  LEU  CYS ...
A T G A A A C A C A T T C C T T T A A C C A C A C T G T G T...
              10                   20                   30...
                                ... VAL  ALA  ILE  SER  ALA  VAL  LEU  LEU  THR  ALA
                                ... G T G G C A A T C T C T G C C G T C T T A T T A A C C G C T
                                ...                  40                   50                   60

 CYS  GLY  GLY  SER  GLY  GLY  SER  ASN  PRO  PRO ...
T G T G G T G G C A G T G G T G G T T C A A A T C C A C C T...
              70                   80                   90...
                                ... ALA  PRO  THR  PRO  ILE  PRO  ASN  ALA  SER  GLY
                                ... G C T C C T A C G C C C A T T C C A A A T G C T A G C G G T
                                ...                 100                  110                  120

 SER  GLY  ASN  THR  GLY  ASN  THR  GLY  ASN  ALA ...
T C A G G T A A T A C T G G C A A C A C T G G T A A T G C T...
              130                  140                  150...
                                ... GLY  GLY  THR  ASP  ASN  THR  ALA  ASN  ALA  GLY
                                ... G G C G G T A C T G A T A A T A C A G C C A A T G C A G G T
                                ...                 160                  170                  180

 ASN  THR  GLY  GLY  THR  ASN  SER  GLY  THR  GLY ...
A A T A C A G G C G G T A C A A A C T C T G G T A C A G G C...
              190                  200                  210...
                                ... SER  ALA  ASN  THR  PRO  GLU  PRO  LYS  TYR  LYS
                                ... A G T G C C A A C A C A C C A G A A C C A A A A T A T A A A
                                ...                 220                  230                  240
```

FIG.2B

```
 ASP  VAL  PRO  THR  ASP  GLU  ASN  LYS  LYS  ASP ...
G A T G T G C C A A C C G A T G A A A A T A A A A A A G A T...
              250                  260                  270...
                                ... GLU  VAL  SER  GLY  ILE  GLN  GLU  PRO  ALA  MET
                                ... G A A G T G T C A G G C A T T C A A G A A C C T G C C A T G
                                ...                  280                  290                  300

 GLY  TYR  GLY  MET  ALA  LEU  SER  LYS  MET  ASN ...
G G T T A T G G C A T G G C T T T G A G T A A A A T G A A T...
              310                  320                  330...
                                ... LEU  HIS  LYS  GLN  GLN  ASP  THR  PRO  LEU  ASP
                                ... C T A C A C A A A C A A C A A G A C A C G C C A T T A G A T
                                ...                  340                  350                  360

 GLU  LYS  ASP  ILE  ILE  THR  LEU  ASP  GLY  LYS ...
G A A A A A G A T A T C A T T A C C T T A G A C G G T A A A...
              370                  380                  390...
                                ... LYS  GLN  VAL  ALA  LYS  GLY  GLU  LYS  SER  PRO
                                ... A A A C A A G T T G C A A A A G G T G A A A A A T C G C C A
                                ...                  400                  410                  420

 LEU  PRO  PHE  SER  LEU  ASP  VAL  GLU  ASN  LYS ...
T T G C C A T T T T C G T T G G A T G T A G A A A A T A A A...
              430                  440                  450...
                                ... LEU  LEU  ASP  GLY  TYR  ILE  ALA  LYS  MET  ASN
                                ... T T G C T T G A T G G C T A T A T A G C A A A A A T G A A T
                                ...                  460                  470                  480
```

FIG.2C

```
 GLU  ALA  ASP  LYS  ASN  ALA  ILE  GLY  ASP  ARG ...
G A A G C G G A T A A A A A T G C C A T T G G T G A C A G A...
              490                  500                  510...
                                ...  ILE  LYS  LYS  ASP  ASN  LYS  ASP  LYS  SER  LEU
                                ... A T T A A G A A A G A T A A T A A A G A C A A G T C A T T A
                                ...                  520                  530                  540

 SER  LYS  ALA  GLU  LEU  ALA  LYS  GLN  ILE  LYS ...
T C T A A A G C A G A G C T T G C C A A A C A A A T C A A A...
              550                  560                  570...
                                ...  GLU  ASP  VAL  ARG  LYS  SER  HIS  GLU  PHE  GLN
                                ... G A A G A T G T G C G T A A A A G C C A T G A G T T T C A G
                                ...                  580                  590                  600

 GLN  VAL  LEU  SER  SER  LEU  LYS  ASN  LYS  ILE ...
C A A G T A T T A T C A T C A C T G A A A A A C A A A A T T...
              610                  620                  630...
                                ...  PHE  HIS  SER  ASN  ASP  GLY  THR  THR  LYS  ALA
                                ... T T T C A T T C A A A T G A T G G A A C A A C C A A A G C A
                                ...                  640                  650                  660

 THR  THR  ARG  ASP  LEU  GLN  TYR  VAL  ASP  TYR
A C C A C A C G A G A T T T A C A A T A T G T T G A T T A T
              670                  680                  690
                                ...  GLY  TYR  TYR  LEU  VAL  ASN  ASP  GLY  ASN  TYR
                                ... G G T T A C T A C T T G G T G A A T G A T G G C A A T T A T
                                ...                  700                  710                  720
```

FIG.2D

```
 LEU  THR  VAL  LYS  THR  ASP  GLU  LEU  TRP  ASN ...
CTAACCGTCAAAACAGACGAACTTTGGAAT
          730            740            750
                    ... LEU  GLY  PRO  VAL  GLY  GLY  VAL  PHE  TYR  ASN
                    ... TTAGGCCCTGTGGGCGGTGTGTTTTATAAT
                    ...          760            770            780

 GLY  THR  THR  THR  ALA  LYS  GLU  LEU  PRO  THR ...
GGCACAACGACCGCCAAAGAGCTACCCACA...
          790            800            810...
                    ... GLN  ASP  ALA  VAL  LYS  TYR  LYS  GLY  HIS  TRP
                    ... CAAGATGCGGTCAAATATAAAGGACATTGG
                    ...          820            830            840

 ASP  PHE  MET  THR  ASP  VAL  ALA  LYS  GLN  ARG ...
GACTTTATGACCGATGTTGCCAAACAAAGA...
          850            860            870...
                    ... ASN  ARG  PHE  SER  GLU  VAL  LYS  GLU  ASN  LEU
                    ... AACCGATTTAGCGAAGTGAAAGAAAACCTT
                    ...          880            890            900

 GLN  ALA  GLY  ARG  TYR  TYR  GLY  ALA  SER  SER ...
CAAGCAGGTCGGTATTATGGAGCATCTTCA...
          910            920            930...
                    ... LYS  ASP  GLU  TYR  ASN  ARG  LEU  LEU  THR  ASP
                    ... AAAGATGAATACAACCGCTTATTAACTGAT
                    ...          940            950            960
```

FIG.2E

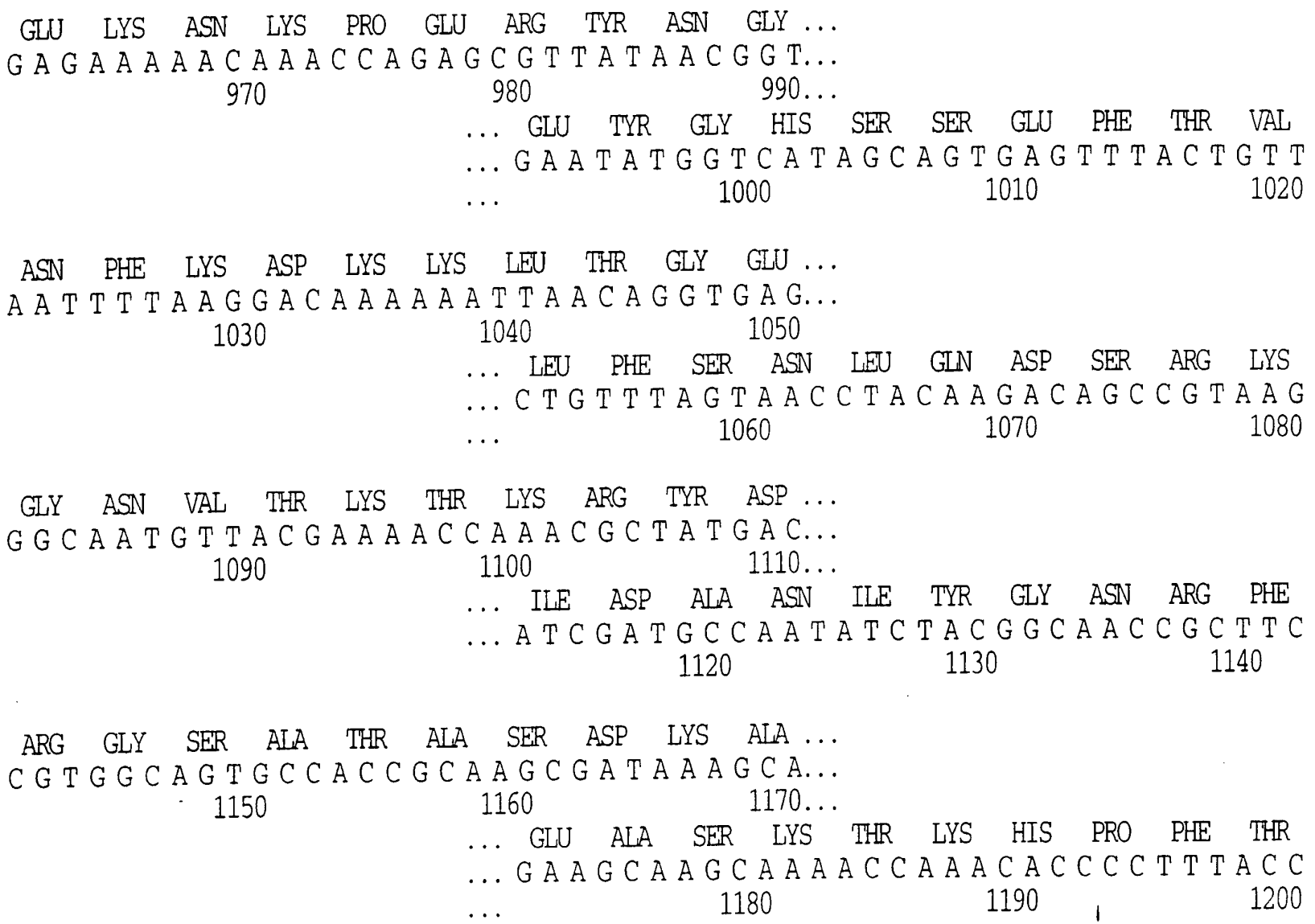

GLU LYS ASN LYS PRO GLU ARG TYR ASN GLY ...
G A G A A A A A C A A A C C A G A G C G T T A T A A C G G T...
970 980 990...
... GLU TYR GLY HIS SER SER GLU PHE THR VAL
... G A A T A T G G T C A T A G C A G T G A G T T T A C T G T T
... 1000 1010 1020
ASN PHE LYS ASP LYS LYS LEU THR GLY GLU ...
A A T T T T A A G G A C A A A A A A T T A A C A G G T G A G...
1030 1040 1050
... LEU PHE SER ASN LEU GLN ASP SER ARG LYS
... C T G T T T A G T A A C C T A C A A G A C A G C C G T A A G
... 1060 1070 1080
GLY ASN VAL THR LYS THR LYS ARG TYR ASP ...
G G C A A T G T T A C G A A A A C C A A A C G C T A T G A C...
1090 1100 1110...
... ILE ASP ALA ASN ILE TYR GLY ASN ARG PHE
... A T C G A T G C C A A T A T C T A C G G C A A C C G C T T C
... 1120 1130 1140
ARG GLY SER ALA THR ALA SER ASP LYS ALA ...
C G T G G C A G T G C C A C C G C A A G C G A T A A A G C A...
1150 1160 1170...
... GLU ALA SER LYS THR LYS HIS PRO PHE THR
... G A A G C A A G C A A A A C C A A A C A C C C C T T T A C C
... 1180 1190 1200

FIG.2F

```
 SER  ASP  ALA  LYS  ASN  SER  LEU  GLU  GLY  GLY ...
A G C G A T G C C A A A A A T A G C C T A G A A G G C G G T...
            1210              1220              1230...
                          ... PHE  TYR  GLY  PRO  ASN  ALA  GLU  GLU  LEU  ALA
                          ... T T T T A T G G A C C A A A C G C C G A G G A G C T G G C A
                          ...           1240              1250              1260

 GLY  LYS  PHE  LEU  THR  ASN  ASP  ASN  LYS  LEU ...
G G T A A A T T C C T A A C C A A T G A C A A C A A A C T C...
            1270              1280              1290...
                          ... PHE  GLY  VAL  PHE  GLY  ALA  LYS  ARG  GLU  SER
                          ... T T T G G C G T C T T T G G T G C T A A A C G A G A G A G T
                          ...           1300              1310              1320

 LYS  ALA  GLY  GLU  LYS  THR  GLU  ALA  ILE  LEU ...
A A A G C T G G G G A A A A A A C C G A A G C C A T C T T A...
            1330              1340              1350
                          ... ASP  ALA  TYR  ALA  LEU  GLY  THR  PHE  ASN  LYS
                          ... G A T G C C T A T G C A C T T G G G A C A T T T A A C A A A
                          ...           1360              1370              1380

 ASN  ASN  ALA  THR  THR  PHE  THR  PRO  PHE  THR ...
A A T A A C G C A A C C A C A T T C A C C C C A T T T A C C...
            1390              1400              1410...
                          ... LYS  LYS  GLN  LEU  ASP  ASN  PHE  GLY  ASN  ALA
                          ... A A A A A A C A A C T G G A T A A C T T T G G C A A T G C C
                          ...           1420              1430              1440
```

FIG.2G

```
 LYS   LYS   LEU   VAL   LEU   GLY   SER   THR   VAL   ILE ...
A A A A A G T T G G T C T T G G G T T C T A C C G T C A T T...
              1450                1460                1470
                                ... ASP   LEU   VAL   PRO   THR   GLY   VAL   THR   LYS   ASP
                                ... G A T T T G G T G C C T A C C G G T G T C A C C A A A G A T
                                ...                 1480                1490                1500

 VAL   ASN   GLU   PHE   THR   LYS   ASN   LYS   PRO   ASP ...
G T C A A T G A A T T C A C C A A A A A C A A G C C A G A T...
              1510                1520                1530
                                ... SER   ALA   THR   ASN   LYS   ALA   GLY   GLU   THR   LEU
                                ... T C T G C C A C A A A C A A A G C G G G C G A G A C T T T G
                                ...                 1540                1550                1560

 MET   VAL   ASN   ASP   LYS   VAL   SER   VAL   LYS   THR ...
A T G G T G A A T G A T A A A G T T A G C G T C A A A A C C...
              1570                1580                1590...
                                ... TYR   GLY   TYR   GLY   ARG   ASN   PHE   GLU   TYR   LEU
                                ... T A T G G C T A T G G C A G A A A C T T T G A A T A C C T A
                                ...                 1600                1610                1620

 LYS   PHE   GLY   GLU   LEU   SER   VAL   GLY   THR   SER ...
A A A T T T G G T G A G C T C A G T G T C G G C A C A A G C...
              1630                1640                1650...
                                ... ASN   SER   VAL   PHE   LEU   GLN   GLY   GLU   ARG   THR
                                ... A A C A G C G T C T T T T T A C A A G G C G A A C G C A C C
                                ...                 1660                1670                1680
```

FIG.2H

```
 ALA  THR  THR  GLY  GLU  LYS  ALA  VAL  PRO  THR ...
G C T A C C A C A G G C G A G A A A G C C G T A C C A A C C...
              1690                 1700                 1710...
                                  ... LYS  GLY  THR  ALA  LYS  TYR  LEU  GLY  ASN  TRP
                                  ... A A A G G C A C A G C C A A A T A T T T G G G G A A C T G G
                                  ...                 1720                 1730                 1740

 VAL  GLY  TYR  ILE  THR  GLY  LYS  ASP  SER  SER ...
G T A G G A T A C A T C A C A G G A A A G G A C T C A T C A...
              1750                 1760                 1770...
                                  ... LYS  SER  PHE  ASN  GLU  ALA  GLN  ASP  VAL  ALA
                                  ... A A A A G C T T T A A T G A G G C C C A A G A T G T T G C T
                                  ...                 1780                 1790                 1800

 ASP  PHE  ASP  ILE  ASP  PHE  GLU  LYS  LYS  SER ...
G A T T T T G A C A T T G A C T T T G A G A A A A A A T C A...
              1810                 1820                 1830...
                                  ... VAL  LYS  GLY  LYS  LEU  THR  THR  LYS  ASP  ARG
                                  ... G T T A A A G G C A A A C T G A C C A C C A A A G A C C G C
                                  ...                 1840                 1850                 1860

 GLN  ASP  PRO  VAL  PHE  ASN  ILE  THR  GLY  ASP ...
C A A G A C C C T G T A T T T A A C A T C A C A G G T G A C...
              1870                 1880                 1890...
                                  ... ILE  ALA  GLY  ASN  GLY  TRP  THR  GLY  LYS  ALA
                                  ... A T C G C A G G C A A T G G C T G G A C A G G C A A A G C C
                                  ...                 1900                 1910                 1920
```

FIG.2I

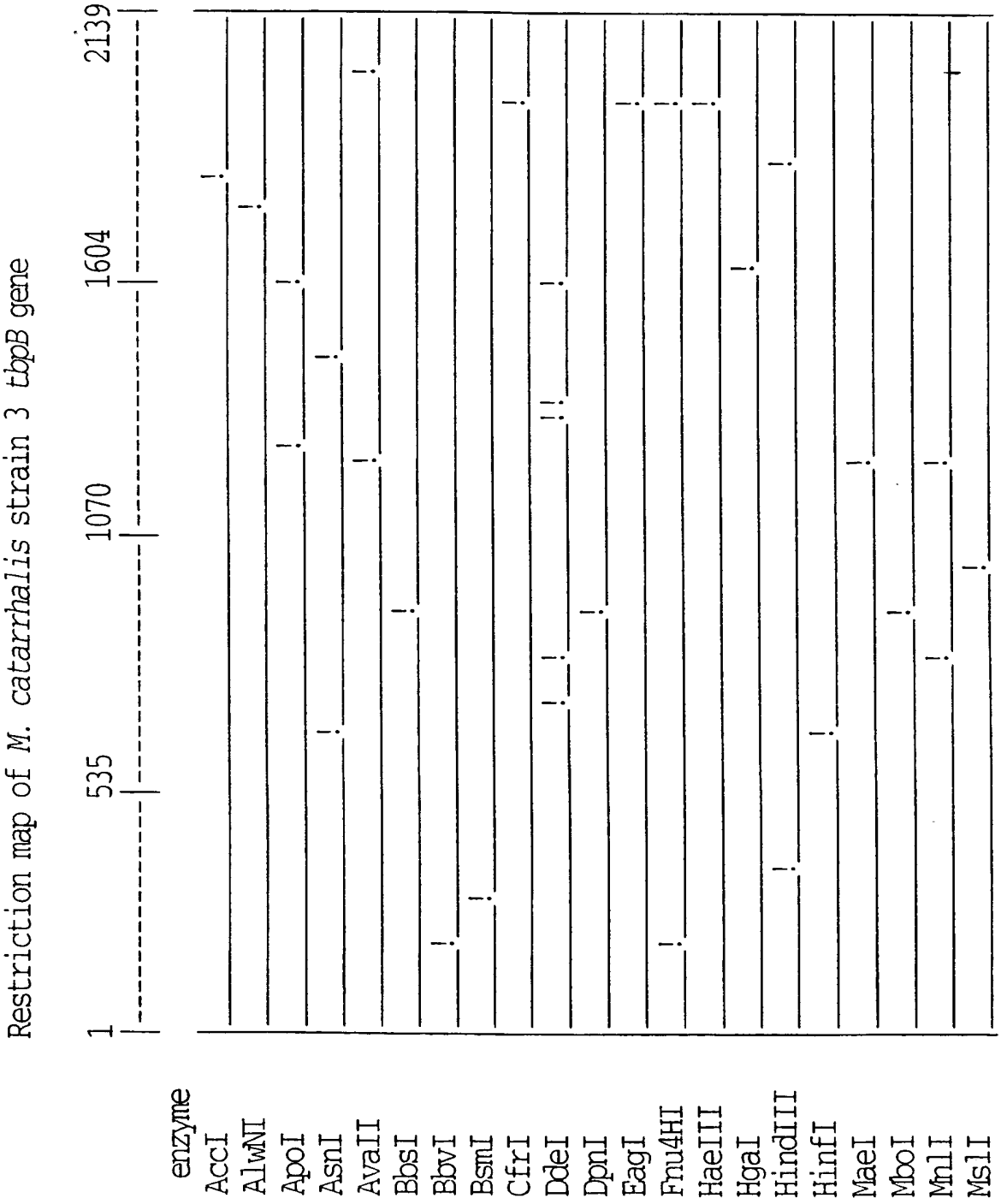
```
 SER  THR  THR  LYS  ALA  ASP  ALA  GLY  GLY  TYR ...
AGCACCACCAAAGCGGACGCAGGGGGCTAC...
          1930           1940           1950...
                                ... LYS  ILE  ASP  SER  SER  SER  THR  GLY  LYS  SER
                                ...AAGATAGATTCTAGCAGTACAGGCAAATCC
                                ...          1960           1970           1980

 ILE  VAL  ILE  LYS  ASP  ALA  GLU  VAL  THR  GLY ...
ATCGTCATCAAAGATGCCGAGGTTACAGGG...
          1990           2000           2010...
                                ... GLY  PHE  TYR  GLY  PRO  ASN  ALA  ASN  GLU  MET
                                ...GGCTTTTATGGTCCAAATGCAAACGAGATG
                                ...          2020           2030           2040

 GLY  GLY  SER  PHE  THR  HIS  ASN  THR  ASP  ASP ...
GGCGGGTCATTTACACACAACACCGATGAC...
          2050           2060           2070...
                                ... SER  LYS  ALA  SER  VAL  VAL  PHE  GLY  THR  LYS
                                ...AGTAAAGCCTCTGTGGTCTTTGGCACAAAA
                                ...          2080           2090           2100

 ARG  GLN  GLU  GLU  VAL  LYS  ***
AGACAAGAAGAAGTTAAGTAG
          2110           2120
```

FIG.3A


Restriction map of M. catarrhalis strain 3 tbpB gene
1
535
1070
1604
2139
enzyme
AccI
AlwNI
ApoI
AsnI
AvaII
BbsI
BbvI
BsmI
CfrI
DdeI
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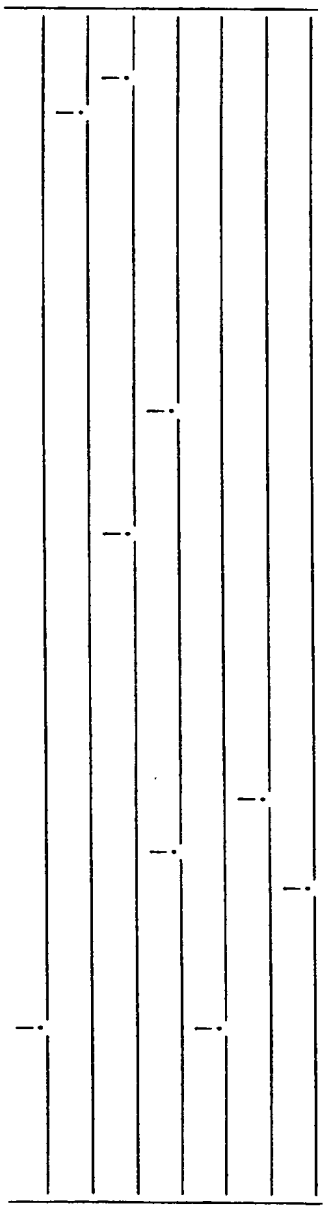


FIG.3B

FIG.4A

M. catarrhalis strain 3 *tbpB* sequence

```
 MET  LYS  HIS  ILE  PRO  LEU  THR  THR  LEU  CYS ...
ATGAAACACATTCCTTTAACCACACTGTGT...
          10                   20                   30...
                                                ... VAL  ALA  ILE  SER  ALA  VAL  LEU  LEU  THR  ALA
                                                ...GTGGCAATCTCTGCCGTCTTATTAACCGCT
                                                ...                  40                   50                   60

 CYS  GLY  GLY  SER  GLY  GLY  SER  ASN  PRO  PRO ...
TGTGGTGGCAGTGGTGGTTCAAATCCACCT...
          70                   80                   90...
                                                ... ALA  PRO  THR  PRO  ILE  PRO  ASN  ALA  GLY  GLY
                                                ...GCTCCTACGCCCATTCCAAATGCAGGCGGT
                                                ...                  100                  110                  120

 ALA  GLY  ASN  ALA  GLY  SER  GLY  THR  GLY  GLY ...
GCAGGTAATGCTGGTAGCGGTACTGGCGGT...
          130                  140                  150...
                                                ... ALA  GLY  SER  THR  ASP  ASN  ALA  ALA  ASN  ALA
                                                ...GCAGGTAGCACTGATAATGCAGCCAATGCA
                                                ...                  160                  170                  180

 GLY  SER  THR  GLY  GLY  ALA  SER  SER  GLY  THR ...
GGCAGTACAGGCGGTGCAAGCTCTGGTACA...
          190                  200                  210...
                                                ... GLY  SER  ALA  SER  THR  GLN  LYS  PRO  LYS  TYR
                                                ...GGCAGTGCCAGCACACAAAAAGCAAAATAT
                                                ...                  220                  230                  240
```

FIG.4B

```
 GLN  ASP  VAL  PRO  THR  ASP  LYS  ASN  LYS  LYS ...
C A A G A T G T G C C A A C C G A T A A A A A T A A A A A A...
              250                  260                  270...
                                ...  ASP  GLU  VAL  SER  GLY  ILE  GLN  GLU  PRO  ALA
                                ... G A T G A A G T G T C A G G C A T T C A A G A A C C T G C C
                                ...                  280                  290                  300

 MET  GLY  TYR  GLY  VAL  GLU  LEU  LYS  LEU  ARG ...
A T G G G T T A T G G C G T G G A A T T A A A G C T T C G T...
              310                  320                  330...
                                ...  ASN  TRP  ILE  PRO  GLN  GLU  GLN  GLU  GLU  HIS
                                ... A A C T G G A T A C C A C A A G A A C A G G A A G A A C A T
                                ...                  340                  350                  360

 ALA  LYS  ILE  ASN  THR  ASN  ASP  VAL  VAL  LYS ...
G C C A A A A T C A A T A C A A A T G A T G T T G T A A A A...
              370                  380                  390...
                                ...  LEU  GLU  GLY  ASP       LEU  LYS  HIS  ASN  PRO  PHE
                                ... C T T G A A G G T G A C T T G A A G C A T A A T C C A T T T
                                ...                  400                  410                  420

 ASP  ASN  SER  ILE  TRP  GLN  ASN  ILE  LYS  ASN ...
G A C A A C T C T A T T T G G C A A A A C A T C A A A A A T...
              430                  440                  450...
                                ...  SER  LYS  GLU  VAL  GLN  THR  VAL  TYR  ASN  GLN
                                ... A G C A A A G A A G T A C A A A C T G T T T A C A A C C A A
                                ...                  460                  470                  480
```

FIG.4C

```
 GLU  LYS  GLN  ASN  ILE  GLU  ASN  GLN  ILE  LYS ...
G A G A A G C A A A A C A T T G A A A A T C A A A T C A A A...
              490                  500                  510...
                                ... LYS  GLU  ASN  LYS  GLU  LEU  ASP  LYS  THR  ALA
                                ... A A A G A A A A T A A A G A A C T T G A T A A A A C G G C A
                                ...                  520                  530                  540

 LEU  LYS  ALA  LEU  ILE  GLU  LYS  VAL  LEU  ASP ...
C T A A A A G C T C T T A T T G A A A A A G T T C T T G A T...
              550                  560                  570...
                                ... ASP  TYR  LEU  THR  SER  LEU  ALA  LYS  PRO  ILE
                                ... G A C T A T C T A A C A A G T C T T G C T A A A C C C A T T
                                ...                  580                  590                  600

 TYR  GLU  LYS  ASN  ILE  ASN  ASP  SER  HIS  ASP ...
T A T G A A A A A A A T A T T A A T G A T T C A C A T G A T...
              610                  620                  630...
                                ... LYS  GLN  ASN  LYS  ALA  ARG  THR  ARG  ASP  LEU
                                ... A A G C A G A A T A A A G C A C G C A C T C G T G A T T T G
                                ...                  640                  650                  660

 LYS  TYR  VAL  ARG  SER  GLY  TYR  ILE  TYR  ARG ...
A A G T A T G T G C G T T C T G G T T A T A T T T A T C G C...
              670                  680                  690...
                                ... SER  GLY  TYR  SER  ASN  ILE  ASP  ILE  GLN  LYS
                                ... T C A G G T T A T T C T A A T A T C G A C A T T C A A A A G
                                ...                  700                  710                  720
```

FIG.4D

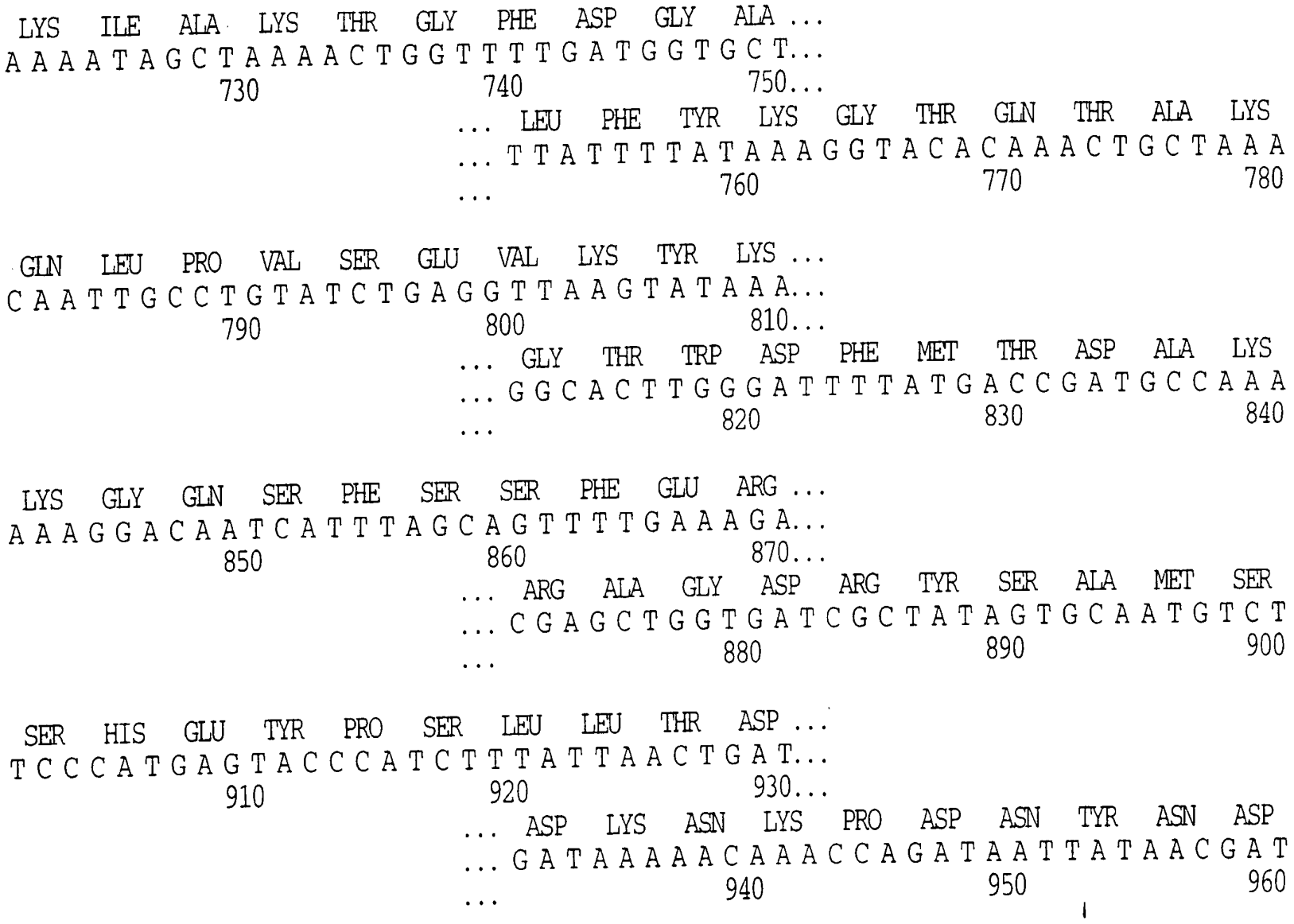

LYS ILE ALA LYS THR GLY PHE ASP GLY ALA ...
A A A A T A G C T A A A A C T G G T T T T G A T G G T G C T...
730 740 750...
... LEU PHE TYR LYS GLY THR GLN THR ALA LYS
... T T A T T T T A T A A A G G T A C A C A A A C T G C T A A A
... 760 770 780
GLN LEU PRO VAL SER GLU VAL LYS TYR LYS ...
C A A T T G C C T G T A T C T G A G G T T A A G T A T A A A...
790 800 810...
... GLY THR TRP ASP PHE MET THR ASP ALA LYS
... G G C A C T T G G G A T T T T A T G A C C G A T G C C A A A
... 820 830 840
LYS GLY GLN SER PHE SER SER PHE GLU ARG ...
A A A G G A C A A T C A T T T A G C A G T T T T G A A A G A...
850 860 870...
... ARG ALA GLY ASP ARG TYR SER ALA MET SER
... C G A G C T G G T G A T C G C T A T A G T G C A A T G T C T
... 880 890 900
SER HIS GLU TYR PRO SER LEU LEU THR ASP ...
T C C C A T G A G T A C C C A T C T T T A T T A A C T G A T...
910 920 930...
... ASP LYS ASN LYS PRO ASP ASN TYR ASN ASP
... G A T A A A A A C A A A C C A G A T A A T T A T A A C G A T
... 940 950 960

FIG.4E

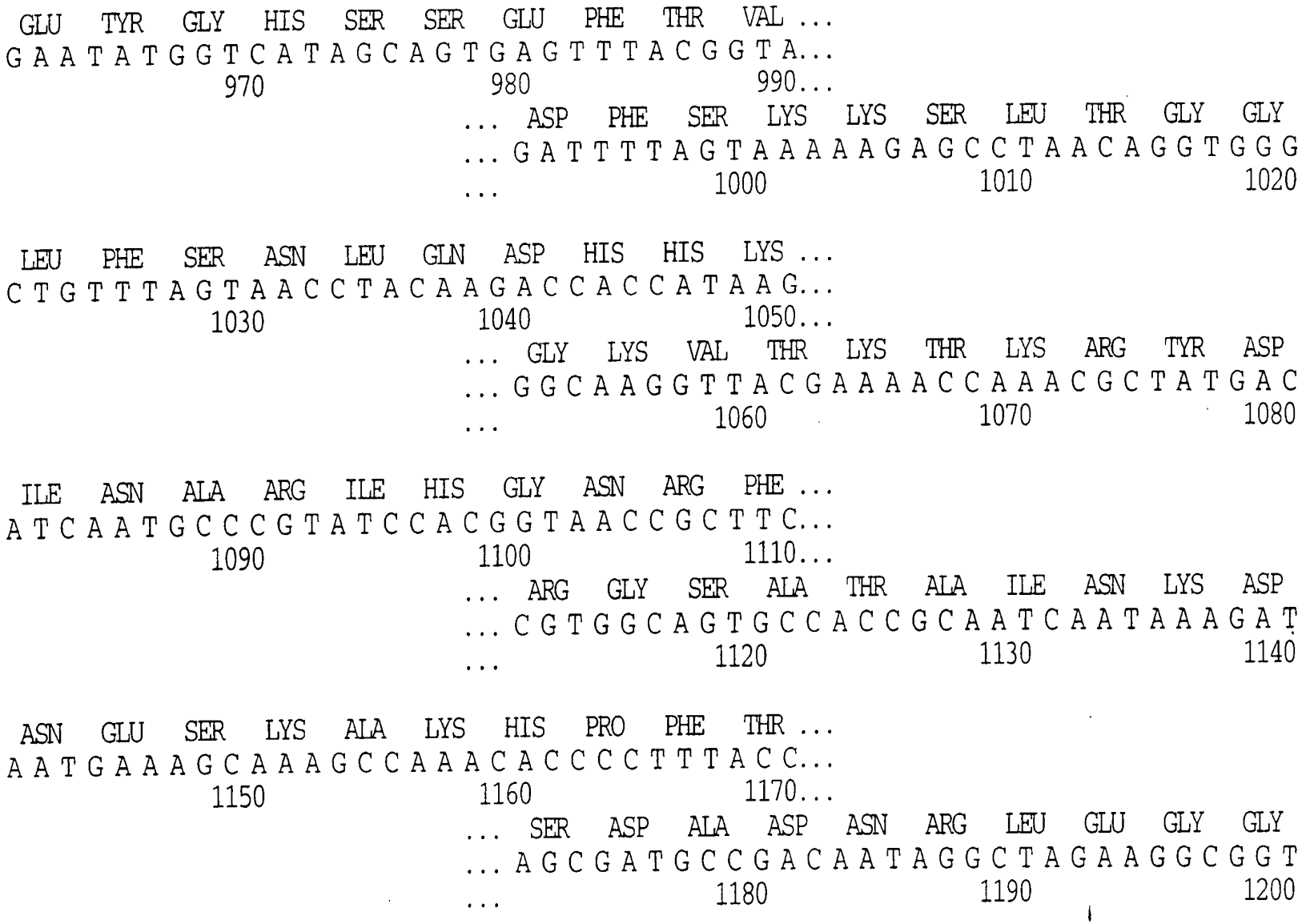

GLU TYR GLY HIS SER SER GLU PHE THR VAL ...
G A A T A T G G T C A T A G C A G T G A G T T T A C G G T A...
970 980 990...
... ASP PHE SER LYS LYS SER LEU THR GLY GLY
... G A T T T T A G T A A A A A G A G C C T A A C A G G T G G G
... 1000 1010 1020
LEU PHE SER ASN LEU GLN ASP HIS HIS LYS ...
C T G T T T A G T A A C C T A C A A G A C C A C C A T A A G...
1030 1040 1050...
... GLY LYS VAL THR LYS THR LYS ARG TYR ASP
... G G C A A G G T T A C G A A A A C C A A A C G C T A T G A C
... 1060 1070 1080
ILE ASN ALA ARG ILE HIS GLY ASN ARG PHE ...
A T C A A T G C C C G T A T C C A C G G T A A C C G C T T C...
1090 1100 1110...
... ARG GLY SER ALA THR ALA ILE ASN LYS ASP
... C G T G G C A G T G C C A C C G C A A T C A A T A A A G A T
... 1120 1130 1140
ASN GLU SER LYS ALA LYS HIS PRO PHE THR ...
A A T G A A A G C A A A G C C A A A C A C C C C T T T A C C...
1150 1160 1170...
... SER ASP ALA ASP ASN ARG LEU GLU GLY GLY
... A G C G A T G C C G A C A A T A G G C T A G A A G G C G G T
... 1180 1190 1200

FIG.4F

```
 PHE  TYR  GLY  PRO  ASN  ALA  GLU  GLU  LEU  ALA ...
T T T T A T G G A C C A A A C G C C G A G G A G C T G G C A...
          1210              1220              1230...
                          ... GLY  LYS  PHE  LEU  THR  ASP  ASP  ASN  LYS  LEU
                          ... G G T A A A T T C C T A A C C G A T G A C A A C A A A C T C
                          ...           1240              1250              1260

 PHE  GLY  VAL  PHE  GLY  ALA  LYS  GLN  GLU  SER ...
T T T G G T G T C T T T G G T G C T A A A C A A G A G A G T...
          1270              1280              1290...
                          ... GLU  ALA  LYS  GLU  THR  GLU  ALA  ILE  LEU  ASP
                          ... G A A G C T A A G G A A A C C G A A G C C A T C T T A G A T
                          ...           1300              1310              1320

 ALA  TYR  ALA  LEU  GLY  THR  PHE  ASN  LYS  SER ...
G C T T A T G C A C T T G G G A C A T T T A A T A A A T C T...
          1330              1340              1350...
                          ... GLY  THR  THR  ASN  PRO  ALA  PHE  THR  ALA  ASN
                          ... G G T A C G A C C A A T C C T G C C T T T A C C G C C A A T
                          ...           1360              1370              1380

 SER  LYS  LYS  GLU  LEU  ASP  ASN  PHE  GLY  ASN ...
A G T A A A A A A G A A C T G G A T A A C T T T G G C A A T...
          1390              1400              1410...
                          ... ILE  ASN  LYS  LEU  VAL  LEU  GLY  SER  THR  VAL
                          ... A T T A A T A A A T T G G T C T T G G G T T C T A C T G T G
                          ...           1420              1430              1440
```

FIG.4G

```
 ILE  ASP  LEU  THR  GLN  GLY  ASN  ASP  PHE  VAL ...
A T A G A C C T T A C T C A A G G T A A T G A T T T T G T A...
            1450              1460              1470...
                             ... LYS  THR  ILE  ASP  LYS  GLU  LYS  PRO  ALA  THR
                             ... A A A A C C A T T G A T A A A G A A A A G C C A G C C A C C
                             ...            1480              1490              1500

 THR  THR  ASN  GLN  ALA  GLY  GLU  PRO  LEU  THR ...
A C T A C C A A T C A A G C A G G C G A G C C T T T G A C G...
            1510              1520              1530...
                             ... VAL  ASN  ASP  LYS  VAL  ARG  VAL  GLN  VAL  CYS
                             ... G T G A A T G A T A A G G T T C G G G T A C A A G T T T G T
                             ...            1540              1550              1560

 CYS  SER  ASN  LEU  GLU  HIS  LEU  LYS  PHE  GLY ...
T G T A G C A A T C T T G A G C A T C T A A A A T T T G G C...
            1570              1580              1590...
                             ... SER  LEU  SER  ILE  GLY  ASP  SER  ASN  SER  VAL
                             ... T C A C T G A G T A T C G G T G A T A G T A A T A G C G T C
                                 ...        1600              1610              1620

 PHE  LEU  GLN  GLY  GLU  ARG  THR  ALA  THR  LYS ...
T T T T T A C A A G G T G A A C G C A C C G C T A C C A A A...
            1630              1640              1650...
                             ... GLY  ASP  LYS  ASP  LYS  ALA  MET  PRO  VAL  ALA
                             ... G G T G A T A A A G A T A A A G C C A T G C C A G T T G C A
                             ...            1660              1670              1680
```

FIG.4H

```
 GLY   ASN   ALA   LYS   TYR   ARG   GLY   THR   TRP   ALA ...
G G A A A T G C T A A A T A C C G T G G T A C A T G G G C A...
              1690                1700                1710...
                                ... GLY   TYR   VAL   ALA   GLY   SER   GLY   ASN   THR   SER
                                ... G G C T A T G T T G C A G G C T C T G G C A A T A C C A G C
                                ...               1720                1730                1740

 LYS   ALA   TYR   GLU   ALA   GLN   GLN   PHE   ALA   ASP ...
A A A G C C T A T G A A G C C C A A C A A T T T G C T G A C...
              1750                1760                1770...
                                ... ASN   ALA   ASN   ARG   ALA   GLU   PHE   ASP   VAL   ASP
                                ... A A T G C C A A C C G T G C C G A G T T T G A T G T A G A C
                                ...               1780                1790                1800

 PHE   ALA   ASN   LYS   SER   LEU   THR   GLY   LYS   LEU ...
T T T G C T A A C A A A A G C C T A A C T G G T A A G C T T...
              1810                1820                1830...
                                ... ILE   PRO   ASN   THR   SER   SER   ASP   GLY   LYS   SER
                                ... A T T C C A A A T A C G A G C A G T G A T G G T A A A T C T
                                ...               1840                1850                1860

 ALA   PHE   ASP   ILE   THR   ALA   THR   ILE   ASP   GLY ...
G C T T T T G A T A T T A C T G C T A C A A T T G A T G G C...
              1870                1880                1890...
                                ... ASN   GLY   PHE   SER   GLY   LYS   ALA   ASN   THR   PRO
                                ... A A T G G T T T T A G T G G T A A A G C C A A T A C A C C A
                                ...               1900                1910                1920
```

FIG.4I

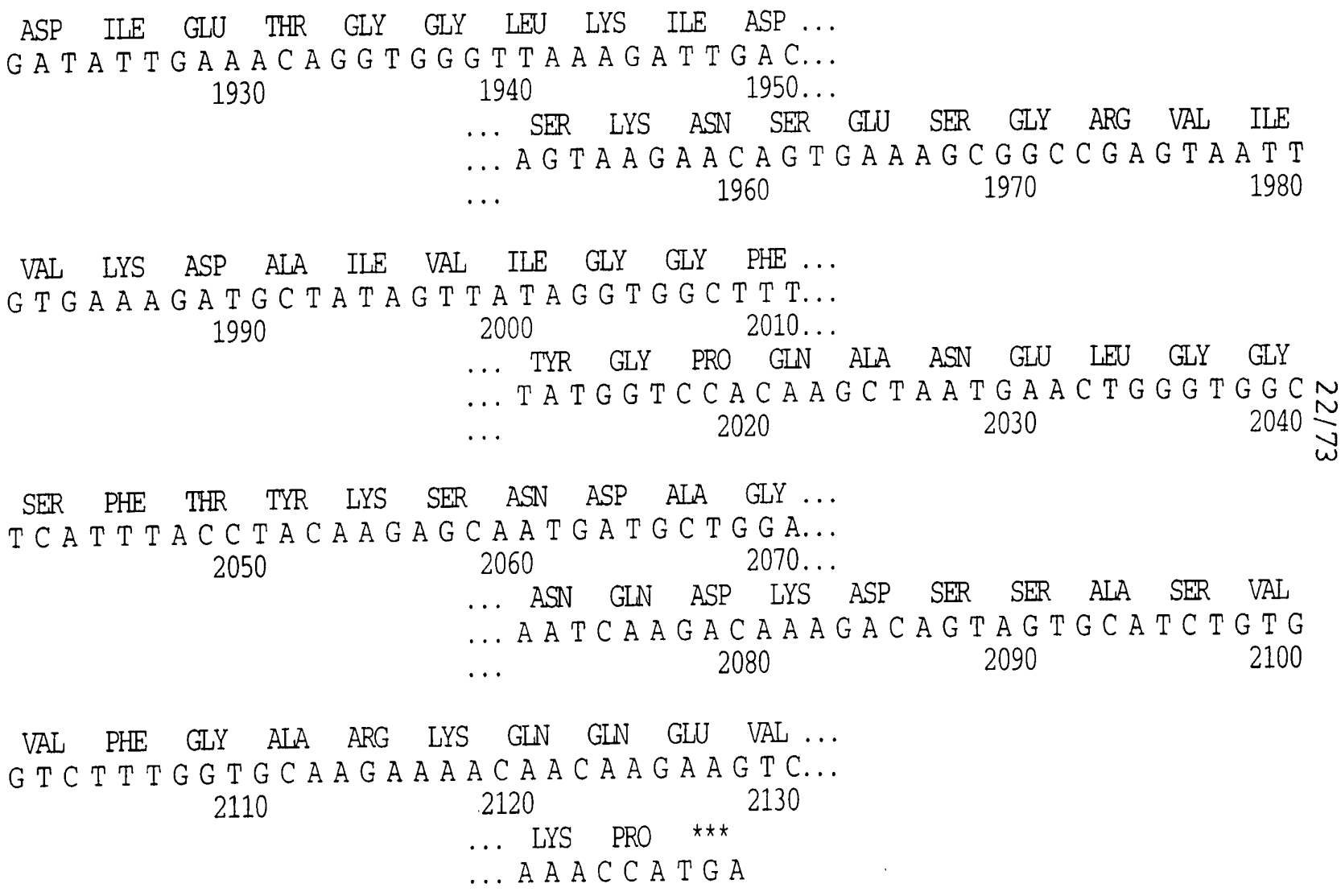
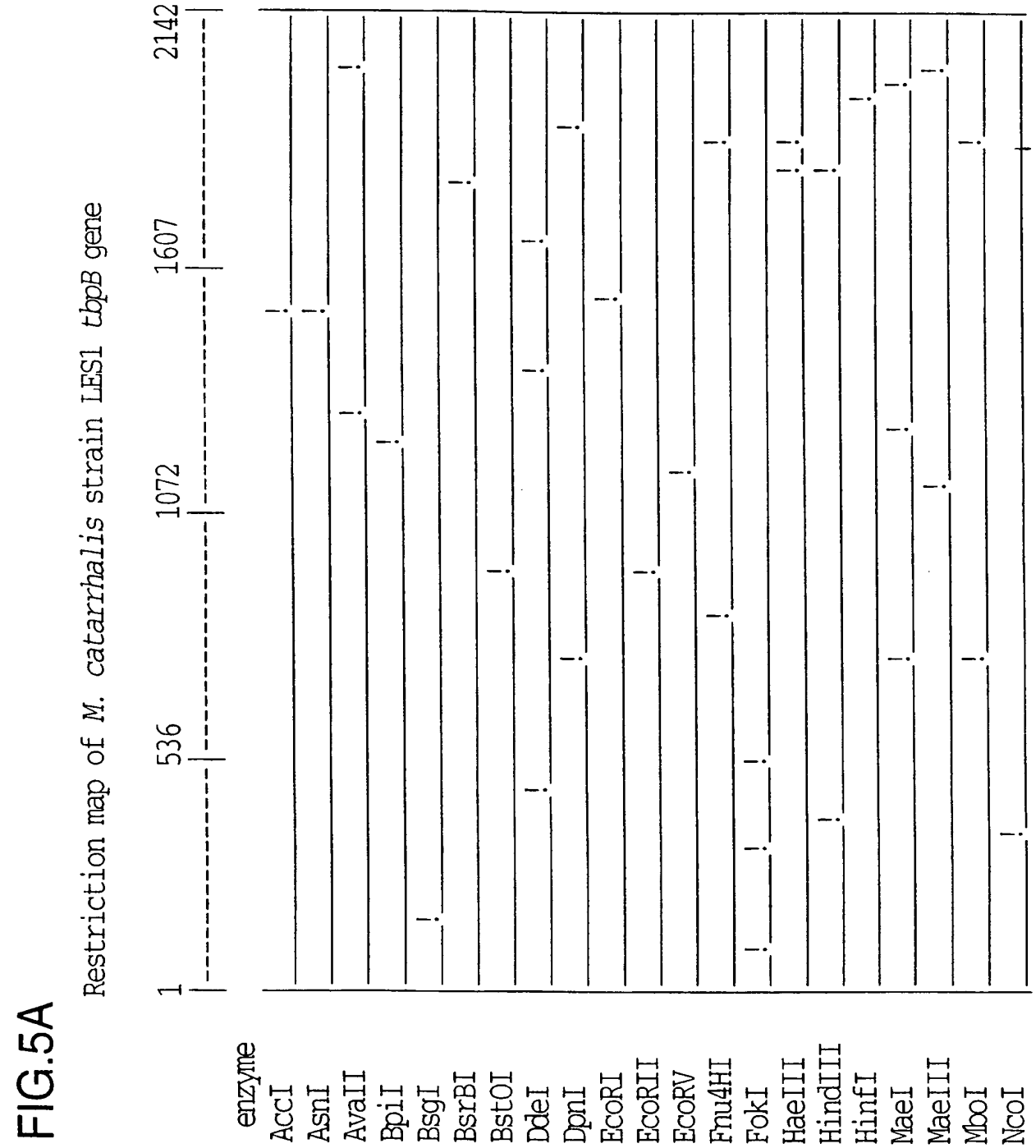


FIG.5A


Restriction map of M. catarrhalis strain LES1 tbpB gene
1
536
1072
1607
2142
enzyme
AccI
AsnI
AvaII
BpiI
BsgI
BsrBI
BstOI
DdeI
DpnI
EcoRI
EcoRII
EcoRV
Fnu4HI
FokI
HaeIII
HindIII
HinfI
MaeI
MaeIII
MboI
NcoI

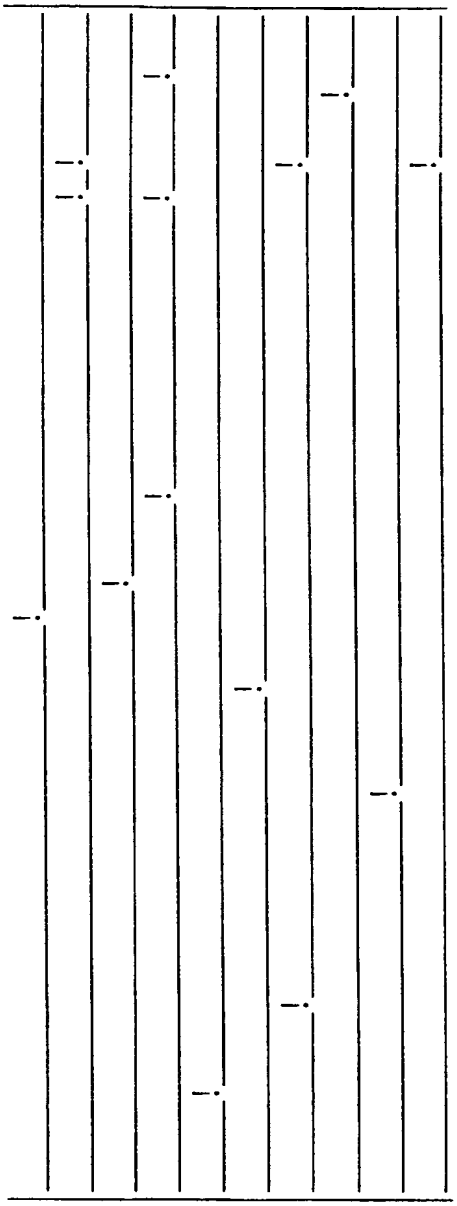


FIG.5B

FIG.6A

M. catarrhalis strain LES1 *tbpB* sequence

```
 MET  LYS  HIS  ILE  PRO  LEU  THR  THR  LEU  CYS ...
A T G A A A C A C A T T C C T T T A A C C A C A C T G T G T...
              10                   20                   30...
                                ... VAL  ALA  ILE  SER  ALA  VAL  LEU  LEU  THR  ALA
                                ... G T G G C A A T C T C T G C C G T C T T A T T A A C C G C T
                                ...                  40                   50                   60

 CYS  GLY  GLY  SER  GLY  GLY  SER  ASN  PRO  PRO ...
T G T G G T G G C A G T G G T G G T T C A A A T C C A C C T...
              70                   80                   90...
                                ... ALA  PRO  THR  PRO  ILE  PRO  ASN  ALA  GLY  SER
                                ... G C T C C T A C G C C C A T C C C A A A T G C A G G C A G T
                                ...                 100                  110                  120

 ALA  GLY  ASN  ALA  GLY  GLY  THR  GLY  ASN  THR ...
G C A G G T A A T G C T G G C G G T A C A G G A A A T A C A...
              130                  140                  150...
                                ... GLY  GLY  THR  GLY  SER  THR  ASP  ASN  VAL  GLY
                                ... G G C G G T A C T G G C A G T A C T G A T A A T G T A G G C
                                ...                 160                  170                  180

 ASN  ALA  GLY  GLY  ALA  ASN  SER  GLY  THR  GLY ...
A A T G C T G G C G G T G C A A A C T C T G G T A C A G G C...
              190                  200                  210...
                                ... ASN  ALA  GLY  ASN  SER  GLY  ASN  ALA  ASN  SER
                                ... A A T G C A G G T A A T T C A G G T A A T G C A A A C T C T
                                ...                 220                  230                  240
```

FIG.6B

```
 GLY  THR  GLY  SER  ALA  ASN  THR  PRO  GLU  PRO ...
G G T A C A G G C A G T G C C A A C A C A C C A G A A C C A...
              250                  260                  270...
                                ... LYS  TYR  GLN  ASP  VAL  PRO  THR  ASP  LYS  ASN
                                ... A A A T A T C A A G A T G T G C C A A C C G A T A A A A A T
                                ...                  280                  290                  300

 GLU  LYS  GLU  GLN  VAL  SER  SER  ILE  GLN  GLU ...
G A A A A A G A A C A A G T T T C A T C C A T T C A A G A A...
              310                  320                  330...
                                ... PRO  ALA  MET  GLY  TYR  ALA  MET  GLU  LEU  LYS
                                ... C C T G C C A T G G G T T A T G C A A T G G A A T T A A A G
                                ...                  340                  350                  360

 LEU  ARG  ASN  ALA  HIS  PRO  LEU  ASN  PRO  ASN ...
C T T C G T A A T G C T C A C C C T C T T A A C C C A A A T...
              370                  380                  390...
                                ... LYS  ASN  LYS  GLU  ALA  GLU  LYS  ARG  ILE  ALA
                                ... A A A A A T A A A G A G G C T G A A A A A C G C A T T G C C
                                ...                  400                  410                  420

 LEU  ASP  GLN  LYS  ASP  LEU  VAL  ALA  VAL  GLU ...
T T A G A C C A A A A A G A T T T G G T G G C A G T A G A G...
              430                  440                  450...
                                ... GLY  ASP  LEU  THR  ASN  ILE  PRO  PHE  ASP  LYS
                                ... G G C G A C C T A A C C A A C A T T C C T T T T G A T A A A
                                ...                  460                  470                  480
```

FIG.6C

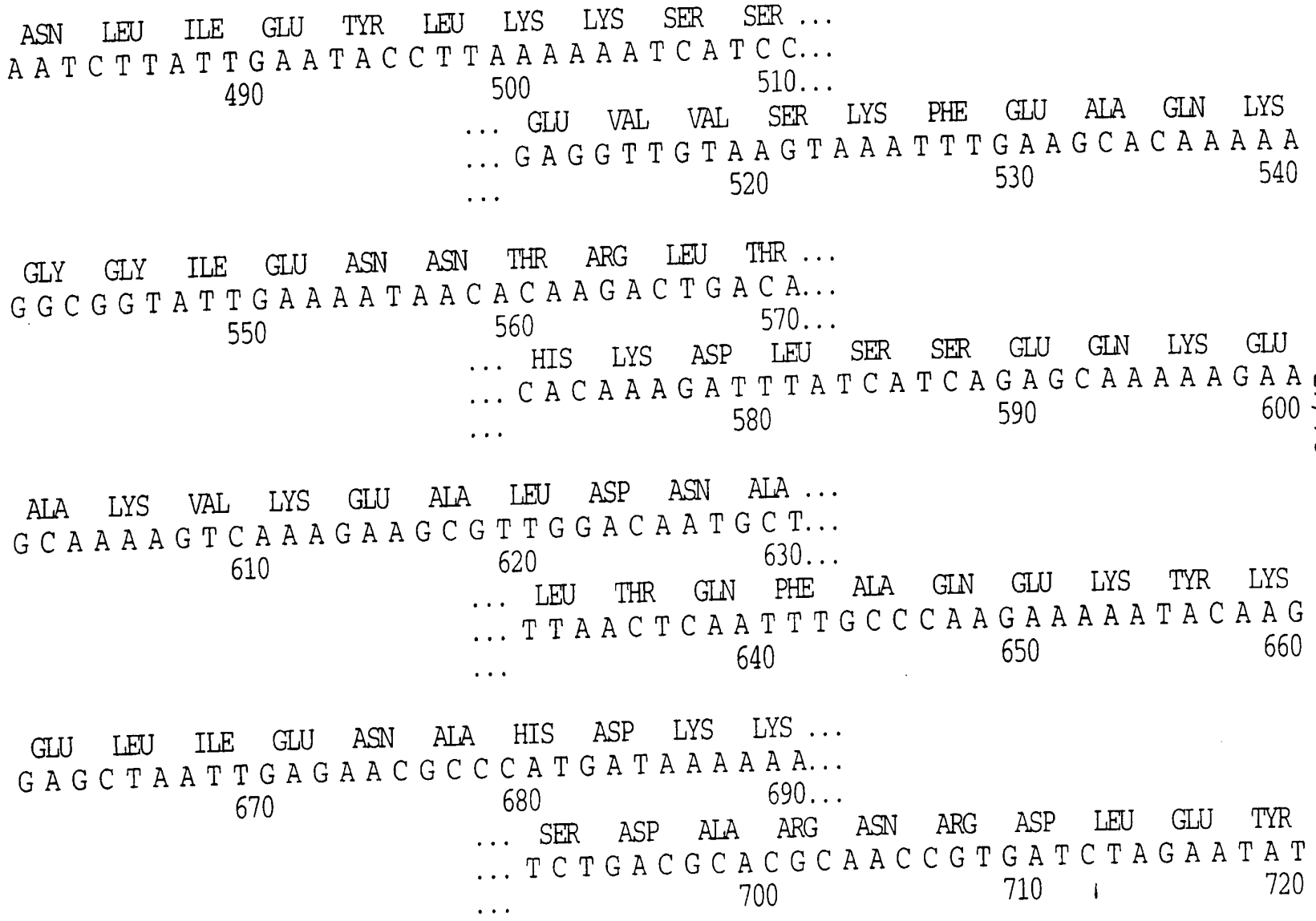


FIG.6D

```
 VAL  LYS  SER  GLY  PHE  ASN  TYR  LEU  SER  GLY ...
G T C A A G T C T G G T T T T A A C T A T C T T T C T G G A...
              730                  740                  750...
                                ...  TYR  THR  ALA  THR  ASP  HIS  ASP  LYS  LYS  THR
                                ... T A T A C C G C C A C C G A C C A C G A C A A A A A A A C C
                                ...                  760                  770                  780

 ASN  TYR  ARG  GLY  TYR  TYR  GLY  ALA  LEU  TYR...
A A T T A T C G T G G C T A T T A T G G T G C G T T G T A T...
              790                  800                  810...
                                ...  TYR  LYS  GLY  SER  GLU  THR  ALA  LYS  GLU  LEU
                                ... T A T A A A G G C A G C G A A A C C G C C A A A G A G C T A
                                ...                  820                  830                  840

 PRO  GLN  THR  SER  ALA  LYS  TYR  LYS  GLY  TYR ...
C C A C A A A C A A G T G C A A A A T A T A A A G G T T A T...
              850                  860                  870...
                                ...  TRP  ASP  PHE  MET  THR  ASP  ALA  THR  LEU  ASP
                                ... T G G G A C T T T A T G A C A    G A T G C C A C A C T T G A T
                                ...                  880                  890                  900

 ASN  LYS  TYR  THR  ASP  LEU  PRO  GLY  ILE  ALA ...
A A C A A A T A C A C G G A T T T G C C A G G T A T C G C C...
              910                  920                  930...
                                ...  ARG  GLN  THR  GLN  TRP  ARG  SER  LEU  VAL  SER
                                ... A G A C A A A C C C A G T G G C G T A G T C T T G T T T C T
                                ...                  940                  950                  960
```

FIG.6E

```
 THR  ASP  GLU  TYR  ALA  THR  LEU  LEU  THR  ASP ...
A C T G A T G A G T A T G C A A C G C T C T T G A C A G A C...
              970                  980                  990...
                                ... LYS  ASN  ASN  LYS  PRO  SER  ASP  TYR  ASN  GLY
                                ... A A A A A T A A C A A G C C C A G T G A T T A C A A T G G T
                                ...                 1000                 1010                 1020

 ALA  TYR  GLY  HIS  SER  SER  GLU  PHE  ASP  VAL ...
G C A T A T G G T C A T A G C A G T G A A T T T G A T G T T...
              1030                 1040                 1050...
                                ... ASN  PHE  ALA  ASP  LYS  LYS  ILE  LYS  GLY  LYS
                                ... A A T T T T G C T G A T A A A A A A A T T A A A G G C A A A
                                ...                 1060                 1070                 1080

 LEU  ILE  SER  ASN  GLN  LEU  SER  GLY  THR  ALA ...
C T T A T C A G T A A T C A G T T A T C A G G C A C A G C T...
              1090                 1100                 1110...
                                ... VAL  THR  ALA  LYS  GLU  ARG  TYR  LYS  ILE  GLU
                                ... G T A A C C G C C A A A G A G C G T T A T A A A A T A G A A
                                ...                 1120                 1130                 1140

 ALA  ASP  ILE  HIS  GLY  ASN  ARG  PHE  ARG  GLY ...
G C T G A T A T C C A C G G C A A C C G C T T C C G T G G C...
              1150                 1160                 1170...
                                ... SER  ALA  THR  ALA  SER  ASP  LYS  ALA  GLU  ASP
                                ... A G T G C C A C C G C A A G C G A T A A A G C A G A A G A C
                                ...                 1180                 1190                 1200
```

FIG.6F

SER LYS THR GLN HIS PRO PHE THR SER ASP ...
AGCAAAACCCAACACCCCTTTACCAGCGAT...
1210 1220 1230...
... ALA THR ASN LYS LEU GLU GLY GLY PHE TYR
... GCTACAAACAAGCTAGAAGGTGGTTTTTAT
... 1240 1250 1260

GLY PRO LYS GLY GLU GLU LEU ALA GLY LYS ...
GGACCAAAAGGCGAGGAGCTGGCAGGTAAA...
1270 1280 1290...
... PHE LEU THR ASP ASP ASN LYS LEU PHE GLY
... TTCTTAACCGATGACAACAAACTCTTTGGG
... 1300 1310 1320

VAL PHE GLY ALA LYS ARG ASP LYS VAL GLU ...
GTCTTTGGTGCTAAACGAGATAAAGTAGAA...
1330 1340 1350...
... LYS THR GLU ALA ILE LEU ASP ALA TYR ALA
... AAAACCGAAGCCATCTTAGATGCCTATGCA
... 1360 1370 1380

LEU GLY THR PHE ASN ASN THR ASN LYS ALA ...
CTTGGGACATTTAATAATACAAATAAAGCA...
1390 1400 1410...
... THR THR PHE THR PRO PHE THR LYS LYS GLN
... ACCACATTCACCCCATTTACCAAAAAACAA
... 1420 1430 1440

FIG.6G

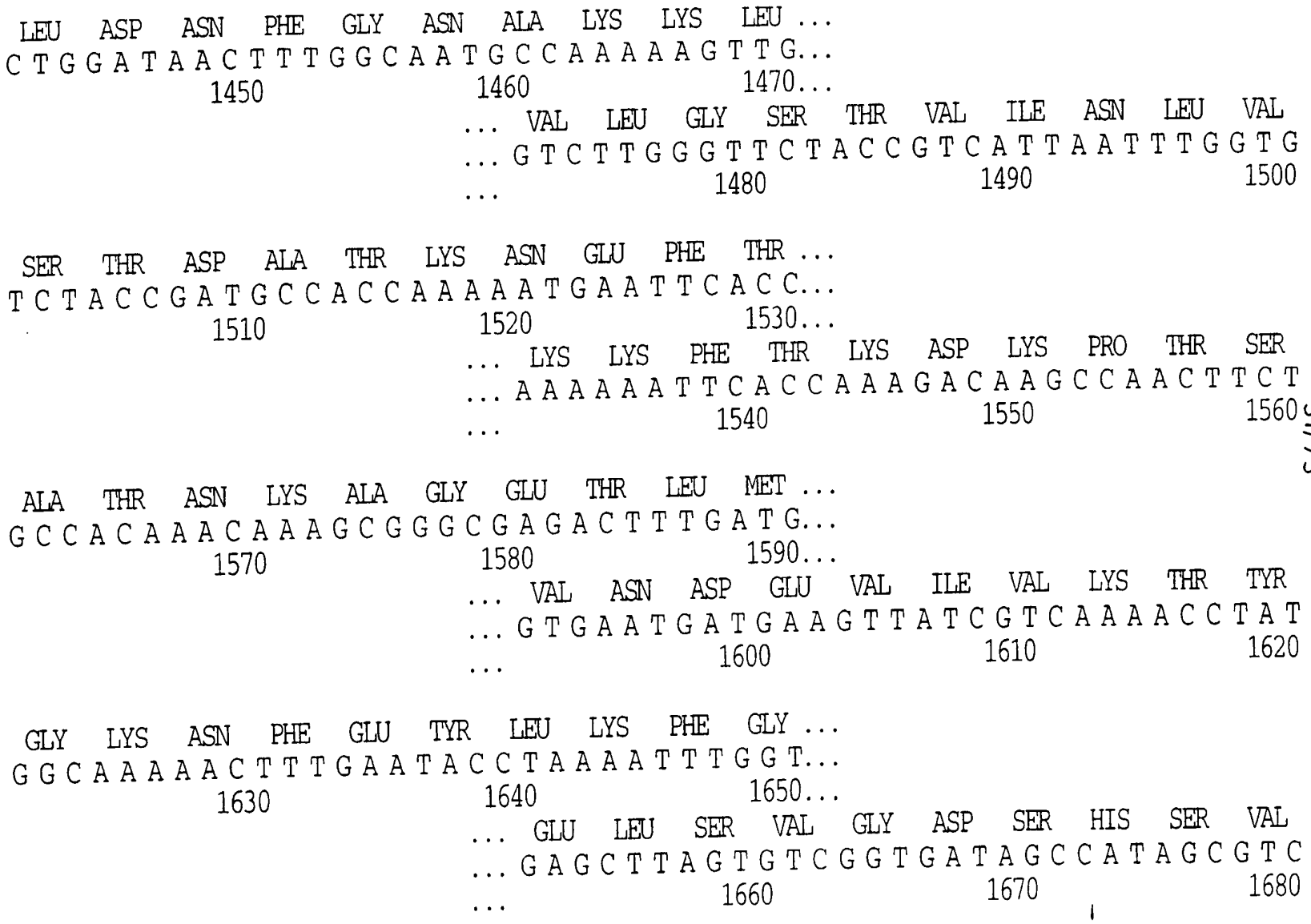

LEU ASP ASN PHE GLY ASN ALA LYS LYS LEU ...
C T G G A T A A C T T T G G C A A T G C C A A A A A G T T G...
1450 1460 1470...
... VAL LEU GLY SER THR VAL ILE ASN LEU VAL
... G T C T T G G G T T C T A C C G T C A T T A A T T T G G T G
... 1480 1490 1500
SER THR ASP ALA THR LYS ASN GLU PHE THR ...
T C T A C C G A T G C C A C C A A A A A T G A A T T C A C C...
1510 1520 1530...
... LYS LYS PHE THR LYS ASP LYS PRO THR SER
... A A A A A A T T C A C C A A A G A C A A G C C A A C T T C T
... 1540 1550 1560
ALA THR ASN LYS ALA GLY GLU THR LEU MET ...
G C C A C A A A C A A A G C G G G C G A G A C T T T G A T G...
1570 1580 1590...
... VAL ASN ASP GLU VAL ILE VAL LYS THR TYR
... G T G A A T G A T G A A G T T A T C G T C A A A A C C T A T
... 1600 1610 1620
GLY LYS ASN PHE GLU TYR LEU LYS PHE GLY ...
G G C A A A A A C T T T G A A T A C C T A A A A T T T G G T...
1630 1640 1650...
... GLU LEU SER VAL GLY ASP SER HIS SER VAL
... G A G C T T A G T G T C G G T G A T A G C C A T A G C G T C
... 1660 1670 1680

FIG.6H

```
 PHE  LEU  GLN  GLY  GLU  ARG  THR  ALA  THR  THR ...
T T T T T A C A A G G C G A A C G C A C C G C T A C C A C A...
          1690                1700                1710...
                          ... GLY  GLU  LYS  ALA  VAL  PRO  THR  THR  GLY  LYS
                          ... G G C G A G A A A G C C G T A C C A A C C A C A G G C A A A
                          ...                1720                1730                1740

 ALA  LYS  TYR  LEU  GLY  ASN  TRP  VAL  GLY  TYR ...
G C C A A A T A T C T G G G G A A C T G G G T A G G A T A C...
          1750                1760                1770...
                          ... ILE  THR  GLY  ALA  GLY  THR  GLY  LYS  SER  PHE
                          ... A T C A C A G G A G C G G G C A C A G G A A A A A G C T T T
                          ...                1780                1790                1800

 ASN  GLU  ALA  GLN  ASP  ILE  ALA  ASP  PHE  ASP ...
A A T G A G G C C C A A G A T A T T G C T G A T T T T G A C...
          1810                1820                1830...
                          ... ILE  ASP  PHE  GLU  ARG  LYS  SER  VAL  LYS  GLY
                          ... A T T G A C T T T G A G A G A A A A T C A G T T A A A G G C
                          ...                1840                1850                1860

 LYS  LEU  THR  THR  GLN  GLY  ARG  THR  ASP  PRO ...
A A A C T G A C C A C C C A A G G C C G C A C A G A T C C T...
          1870                1880                1890...
                          ... VAL  PHE  ASN  ILE  LYS  GLY  GLU  ILE  ALA  GLY
                          ... G T C T T T A A C A T C A A A G G T G A A A T T G C A G G C
                          ...                1900                1910                1920
```

FIG.6I

```
 ASN  GLY  TRP  THR  GLY  LYS  ALA  SER  THR  THR ...
AATGGCTGGACAGGCAAAGCCAGCACCACC...
          1930           1940           1950...
                    ... LYS  ALA  ASP  ALA  GLY  GLY  TYR  LYS  ILE  ASP
                    ... AAAGCGGACGCAGGAGGCTACAAGATAGAT
                    ...          1960           1970           1980

 SER  SER  SER  THR  GLY  LYS  SER  ILE  VAL  ILE ...
TCTAGCAGTACAGGCAAATCCATCGTCATC...
          1990           2000           2010...
                    ... GLU  ASN  ALA  GLU  VAL  THR  GLY  GLY  PHE  TYR
                    ... GAAAATGCCGAAGTTACTGGGGGCTTTTAT
                    ...          2020           2030           2040

 GLY  PRO  ASN  ALA  ASN  GLU  MET  GLY  GLY  SER ...
GGTCCAAATGCAAACGAGATGGGCGGGTCA...
          2050           2060           2070...
                    ... PHE  THR  HIS  ASP  THR  ASP  ASP  SER  LYS  ALA
                    ... TTTACACACGATACCGATGACAGTAAAGCC
                    ...          2080           2090           2100

 SER  VAL  VAL  PHE  GLY  THR  LYS  ARG  GLN  GLN ...
TCTGTGGTCTTTGGCACAAAAAGACAACAA...
          2110           2120           2130...
                    ... GLU  VAL  LYS  ***
                    ... GAAGTTAAGTAG
                    ...          2140
```

FIG.7A Alignment of *M. catarrhalis* TbpB protein sequences

```
10         20         30         40         50         60
MKHIPLTTLCVAISAVLLTACGGSGGS-NPPAPTPIPNASGSGNTGN-TGNAGGTDNTAN
.............................-....................-............
.............................-....................-............
.............................-...........GSA..A.G-...T...GS.D.
.........................S.GF....S......GN...A..-A......GGANS
.............................-...........G.A..A.SG..G..S...A..
                                          70         80         90         100
                                  -AGNTGGT-------------NSGTGSANTPEPKYQDVPTEKNEKDKVSSIQEPAM   4223
                                  -.......-------------S......S.S.........TP.N.EQ.........   R1
                                  -.......-------------..............K....DE.K..E..G......   M35
                                  -V..A..ANSGTGNAGNSGNA....................D....EQ.........   LES1
                                  G...A...----------GGA...A...S......K....DE.K.AE..G......   Q8
                                  -..S...A-------------S......S.QK........D..K..E..G......   3

       110        120        130        140        150
GYGMALSKINLHNRQDTPLDEKNI-ITLDGKKQVA-EGKKSPLPFSLDVENKLLDGYIA
...........YDQ......A...-...........-DNQ....................
........M...KQ........D.-...........-K.E....................
..A.E.KLR.A.PLNPNKNK.AEKR.A..Q.DL..V..DLTNI..DKNLIEY.KKSSEV
...VE.KLR.WIPQ------.QEEHAKINTNDV.KL..DLKHN..DNSIWQNIKNSKEV
...VE.KLR.WIPQ------.QEEHAKINTNDV.KLEGDLKHN..DNSIWQNIKNSKEV
                                  160        170          180         190        200
                                  KMNV-ADKNAIGDRIKKG---NKEISDE--ELAKQIKEAVRKSHEFQQV--       4223
                                  ...E-.......E...RENEQ..K....--....K...N....P.....--       R1
                                  ...E-............D---..DK.LSKA........D..........--       M35
                                  VSKFE.Q.GG.ENNTRLT---H.DL.S.QKE-..-V...---LDNALT.FA       LES1
                                  QTVYNQE.QN.EDQ..RE---..QRP.KK.DDVALQAYIEKVLDDRLTELA       Q8
                                  QTVYNQE.QN.ENQ...E---...-----LDKTALKALIEKVLDDYLTSLA       3
```

FIG.7B

```
        210        220        230        240        250
LSSLENKIFHSNDGTTKATTRDLKYVDYGYYLANDGNYLTVKTD--KLWNLGPVGGVFY
...IKA.T.....K.................V..A.........NP....S........
....K.....................Q.........V............--E.........
QEKYKEL.ENAH.KKSD.RN...E..KS.FNYLSGYTATDHDK---.TNYR.YY.ALY.
KPIY.KN.NY.H.KQN..R.......RS..IYRSGYSNIIP.----.IAKT.FD.AL..
KPIY.KN.ND.H.KQN..R.......RS..IYRSGYSNIDIQK---.IAKT.FD.AL..

                                  260        270        280        290        300
                                  NGTTTAKELPTQDAVKYKGHWDFMTDVANRRNRFSEVKENSQA
                                  ..S.........................KK.........TY..
                                  ............................KQ.........L..
                                  K.SE......QTS.-....Y......ATLDNKYTDLPGIAR.T
                                  Q..Q...Q..VSQ-.....T......AKKGQSFS.FGTSQRL.
                                  K..Q...Q..VSE-.....T......AKKGQSFS.FERRAGDR

        310        320        330        340        350
GWYYGASSKDEYNRLLTKEDSAPDGHSGEYGHSSEFTVNFKEKKLTGKLFSNLQDRH
..W..............A.A...NY.....................E.......S.
.R..............D.KNK.ERYN.............D.....E.......SR
Q.-RSLV.T...AT...DKNNK.SDYN.A.......D...AD..IK...I..QLSG-
.DR.S.M.YH..PS...D.KNK..NYN...........D.SK.S.K.E.S..I..G.
--.SAM..H-..PS...DDKNK..NYND..........D.SK.S...G.......H.

                                  360        370        380        390        400
                                  KGNVTKTERYDIDANIHGNRFRGSATASNKNDTSK--HPFTSDAN
                                  .QK....K....K.D.............D.AED..SK.......K
                                  .......K........Y...........D.AEA..TK.......K
                                  -TA..AK...K.E.D.............D.AED..TQ.......T
                                  ..S.N..K........Y...........DTTEA..SK.......K
                                  ..K....K....N.R............I..DNE..AK.......D
```

4223
R1
M35
LES1
Q8
3

FIG.7C

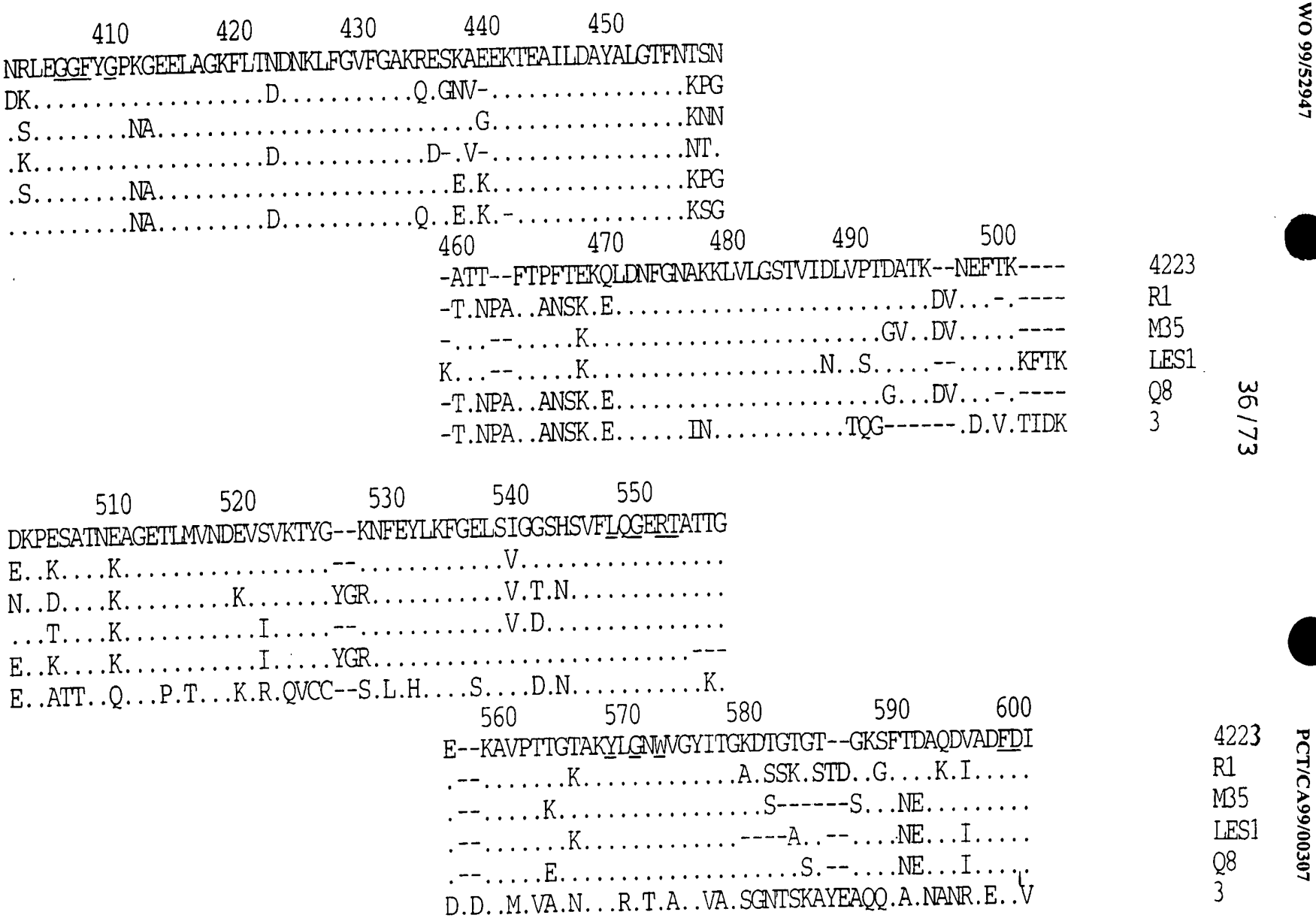


FIG.7D

```
      610       620       630       640       650
DFGNKSVSGKLITKGRQDPV--FSITGQIAGNGWTGTASTTKADAGGYKIDSSSTGKS
..EK...N...T..D.....--.N...E........K...AE.N.............
..EK...K...T..D.....--.N...D........K.....................
..ER...K...T.Q..T...--.N.K.E........K.....................
..ER...K...T.Q......--.N.................A..NV............
..A...LT....PNTSS.GKSA.D..AT.D...FS.K.N.PDIET..L....KNSESG
                                660       670       680                690       700
                     -IVIKDANVTGGFYGPNANEMGGSFTHNA-------DDSKASVVFGTKRQQEVK-*      4223
                     -......V..........T.........S------GN.G.V.......K.-...K*      R1
                     -......E.....................T-------.............E...-*      M35
                     -...EN.E.....................DT--------.................*      LES1
                     -...EN.K.....................DT-------.............E...-*      Q8
                     RVIV...I.I......Q...L.....YKSNDAGNQDK..S......ARK.....P*      3
```

FIG.8A

M. catarrhalis strain 4223 *tbpA* - *orf3* - *tbpB* locus gene sequences

```
G A T G C C T G C C T T G T G A T T G G T T G G G G T G T A...
               10                  20                  30...
                                ... T C G G T G T A T C A A A G T G C A A A A G C C A A C A G G
                                ...                 40                  50                  60

                     tbpA
                     MET   ASN   GLN   SER   LYS   GLN   ASN ...
T G G T C A T T G A T G A A T C A A T C A A A A C A A A A A C...
               70                  80                  90...

                                ...  ASN   LYS   SER   LYS   LYS   SER   LYS   GLN   VAL   LEU
                                ... A A C A A A T C C A A A A A A T C C A A A C A A G T A T T A
                                ...                 100                 110                 120

 LYS   LEU   SER   ALA   LEU   SER   LEU   GLY   LEU   LEU ...
A A A C T T A G T G C C T T G T C T T T G G G T C T G C T T...
               130                 140                 150...
                                ...  ASN   ILE   THR   GLN   VAL   ALA   LEU   ALA   ASN   THR
                                ... A A C A T C A C G C A G G T G G C A C T G G C A A A C A C A
                                ...                 160                 170                 180

 THR   ALA   ASP   LYS   ALA   GLU   ALA   THR   ASP   LYS ...
A C G G C C G A T A A G G C G G A G G C A A C A G A T A A G...
               190                 200                 210...
                                ...  THR   ASN   LEU   VAL   VAL   VAL   LEU   ASP   GLU   THR
                                ... A C A A A C C T T G T T G T T G T C T T G G A T G A A A C T
                                ...                 220                 230                 240
```

FIG.8B

```
 VAL  VAL  THR  ALA  LYS  LYS  ASN  ALA  ARG  LYS ...
G T T G T A A C A G C G A A G A A A A A C G C C C G T A A A...
              250                  260                  270...
                                ... ALA  ASN  GLU  VAL  THR  GLY  LEU  GLY  LYS  VAL
                                ... G C C A A C G A A G T T A C A G G G C T T G G T A A G G T G
                                ...                  280                  290                  300

 VAL  LYS  THR  ALA  GLU  THR  ILE  ASN  LYS  GLU ...
G T C A A A A C T G C C G A G A C C A T C A A T A A A G A A...
              310                  320                  330...
                                ... GLN  VAL  LEU  ASN  ILE  ARG  ASP  LEU  THR  ARG
                                ... C A A G T G C T A A A C A T T C G A G A C T T A A C A C G C
                                ...                  340                  350                  360

 TYR  ASP  PRO  GLY  ILE  ALA  VAL  VAL  GLU  GLN ...
T A T G A C C C T G G C A T T G C T G T G G T T G A G C A A...
              370                  380                  390...
                                ... GLY  ARG  GLY  ALA  SER  SER  GLY  TYR  SER  ILE
                                ... G G T C G T G G G G C A A G C T C A G G C T A T T C T A T T
                                ...                  400                  410                  420

 ARG  GLY  MET  ASP  LYS  ASN  ARG  VAL  ALA  VAL ...
C G T G G T A T G G A T A A A A A T C G T G T G G C G G T A...
              430                  440                  450...
                                ... LEU  VAL  ASP  GLY  ILE  ASN  GLN  ALA  GLN  HIS
                                ... T T G G T T G A T G G C A T C A A T C A A G C C C A G C A C
                                ...                  460                  470                  480
```

FIG.8C

```
 TYR  ALA  LEU  GLN  GLY  PRO  VAL  ALA  GLY  LYS ...
T A T G C C C T A C A A G G C C C T G T G G C A G G C A A A...
              490                  500                  510...
                                                        ... ASN  TYR  ALA  ALA  GLY  GLY  ALA  ILE  ASN  GLU
                                                        ... A A T T A T G C C G C A G G T G G G G C A A T C A A C G A A
                                                        ...                  520                  530                  540

 ILE  GLU  TYR  GLU  ASN  VAL  ARG  SER  VAL  GLU ...
A T A G A A T A C G A A A A T G T C C G C T C C G T T G A G...
              550                  560                  570...
                                                        ... ILE  SER  LYS  GLY  ALA  ASN  SER  SER  GLU  TYR
                                                        ... A T T A G T A A A G G T G C A A A T T C A A G T G A A T A C
                                                        ...                  580                  590                  600

 GLY  SER  GLY  ALA  LEU  SER  GLY  SER  VAL  ALA ...
G G C T C T G G G G C A T T A T C T G G C T C T G T G G C A...
              610                  620                  630...
                                                        ... PHE  VAL  THR  LYS  THR  ALA  ASP  ASP  ILE  ILE
                                                        ... T T T G T T A C C A A A A C C G C C G A T G A C A T C A T C
                                                        ...                  640                  650                  660

 LYS  ASP  GLY  LYS  ASP  TRP  GLY  VAL  GLN  THR ...
A A A G A T G G T A A A G A T T G G G G C G T G C A G A C C...
              670                  680                  690...
                                                        ... LYS  THR  ALA  TYR  ALA  SER  LYS  ASN  ASN  ALA
                                                        ... A A A A C C G C C T A T G C C A G T A A A A A T A A C G C A
                                                        ...                  700                  710                  720
```

FIG.8D

```
 TRP  VAL  ASN  SER  VAL  ALA  ALA  ALA  GLY  LYS ...
T G G G T T A A T T C T G T G G C A G C A G C A G G C A A G...
              730                  740                  750...
                                ... ALA  GLY  SER  PHE  SER  GLY  LEU  ILE  ILE  TYR
                                ... G C A G G T T C T T T T A G C G G T C T T A T C A T C T A C
                                ...                  760                  770                  780
                                ...

 THR  ASP  ARG  ARG  GLY  GLN  GLU  TYR  LYS  ALA ...
A C C G A C C G C C G T G G T C A A G A A T A C A A G G C A...
              790                  800                  810...
                                ... HIS  ASP  ASP  ALA  TYR  GLN  GLY  SER  GLN  SER
                                ... C A T G A T G A T G C C T A T C A G G G T A G C C A A A G T
                                ...                  820                  830                  840
                                ...

 PHE  ASP  ARG  ALA  VAL  ALA  THR  THR  ASP  PRO ...
T T T G A T A G A G C G G T G G C A A C C A C T G A C C C A...
              850                  860                  870...
                                ... ASN  ASN  ARG  THR  PHE  LEU  ILE  ALA  ASN  GLU
                                ... A A T A A C C G A A C A T T T T T A A T A G C A A A T G A A
                                ...                  880                  890                  900
                                ...

 CYS  ALA  ASN  GLY  ASN  TYR  GLU  ALA  CYS  ALA ...
T G T G C C A A T G G T A A T T A T G A G G C G T G T G C T...
              910                  920                  930...
                                ... ALA  GLY  GLY  GLN  THR  LYS  LEU  GLN  ALA  LYS
                                ... G C T G G C G G T C A A A C C A A A C T T C A A G C C A A G
                                ...                  940                  950                  960
                                ...
```

FIG.8E

```
 PRO  THR  ASN  VAL  ARG  ASP  LYS  VAL  ASN  VAL ...
C C A A C C A A T G T G C G T G A T A A G G T C A A T G T C...
              970                  980                  990...
                                ... LYS  ASP  TYR  THR  GLY  PRO  ASN  ARG  LEU  ILE
                                ... A A A G A T T A T A C A G G T C C T A A C C G C C T T A T C
                                ...              1000                 1010                 1020

 PRO  ASN  PRO  LEU  THR  GLN  ASP  SER  LYS  SER ...
C C A A A C C C A C T C A C C C A A G A C A G C A A A T C C...
              1030                 1040                 1050...
                                ... LEU  LEU  LEU  ARG  PRO  GLY  TYR  GLN  LEU  ASN
                                ... T T A C T G C T T C G C C C A G G T T A T C A G C T A A A C
                                ...              1060                 1070                 1080

 ASP  LYS  HIS  TYR  VAL  GLY  GLY  VAL  TYR  GLU ...
G A T A A G C A C T A T G T C G G T G G T G T G T A T G A A...
              1090                 1100                 1110...
                                ... ILE  THR  LYS  GLN  ASN  TYR  ALA  MET  GLN  ASP
                                ... A T C A C C A A A C A A A A C T A C G C C A T G C A A G A T
                                ...              1120                 1130                 1140

 LYS  THR  VAL  PRO  ALA  TYR  LEU  ALA  VAL  HIS ...
A A A A C C G T G C C T G C T T A T C T G G C G G T T C A T...
              1150                 1160                 1170...
                                ... ASP  ILE  GLU  LYS  SER  ARG  LEU  SER  ASN  HIS
                                ... G A C A T T G A A A A A T C A A G G C T C A G C A A C C A T
                                ...              1180                 1190                 1200
```

FIG.8F

```
 ALA  GLN  ALA  ASN  GLY  TYR  TYR  GLN  GLY  ASN ...
G C C C A A G C C A A T G G C T A T T A T C A A G G C A A T...
          1210           1220           1230...
                    ... ASN  LEU  GLY  GLU  ARG  ILE  ARG  ASP  THR  ILE
                    ... A A T C T T G G T G A A C G C A T T C G T G A T A C C A T T
                    ...          1240           1250           1260

 GLY  PRO  ASP  SER  GLY  TYR  GLY  ILE  ASN  TYR ...
G G G C C A G A T T C A G G T T A T G G C A T C A A C T A T...
          1270           1280           1290...
                    ... ALA  HIS  GLY  VAL  PHE  TYR  ASP  GLU  LYS  HIS
                    ... G C T C A T G G C G T A T T T T A T G A T G A A A A A C A C
                    ...          1300           1310           1320

 GLN  LYS  ASP  ARG  LEU  GLY  LEU  GLU  TYR  VAL ...
C A A A A A G A C C G C C T A G G G C T T G A A T A T G T T...
          1330           1340           1350...
                    ... TYR  ASP  SER  LYS  GLY  GLU  ASN  LYS  TRP  PHE
                    ... T A T G A C A G C A A A G G T G A A A A T A A A T G G T T T
                    ...          1360           1370           1380

 ASP  ASP  VAL  ARG  VAL  SER  TYR  ASP  LYS  GLN ...
G A T G A T G T G C G T G T G T C T T A T G A T A A G C A A...
          1390           1400           1410...
                    ... ASP  ILE  THR  LEU  ARG  SER  GLN  LEU  THR  ASN
                    ... G A C A T T A C G C T A C G C A G C C A G C T G A C C A A C
                    ...          1420           1430           1440
```

FIG.8G

```
 THR  HIS  CYS  SER  THR  TYR  PRO  HIS  ILE  ASP ...
A C G C A C T G T T C A A C C T A T C C G C A C A T T G A C...
          1450                 1460                 1470...
                                ... LYS  ASN  CYS  THR  PRO  ASP  VAL  ASN  LYS  PRO
                                ...A A A A A T T G T A C G C C T G A T G T C A A T A A A C C T
                                ...                 1480                 1490                 1500
                                ...

 PHE  SER  VAL  LYS  GLU  VAL  ASP  ASN  ASN  ALA ...
T T T T C G G T A A A A G A G G T G G A T A A C A A T G C C...
          1510                 1520                 1530...
                                ... TYR  LYS  GLU  GLN  HIS  ASN  LEU  ILE  LYS  ALA
                                ... T A C A A A G A A C A G C A C A A T T T A A T C A A A G C C
                                ...                 1540                 1550                 1560
                                ...

 VAL  PHE  ASN  LYS  LYS  MET  ALA  LEU  GLY  SER ...
G T C T T T A A C A A A A A A A T G G C G T T G G G C A G T...
          1570                 1580                 1590...
                                ... THR  HIS  HIS  HIS  ILE  ASN  LEU  GLN  VAL  GLY
                                ... A C G C A T C A T C A C A T C A A C C T G C A A G T T G G C
                                ...                 1600                 1610                 1620
                                ...

 TYR  ASP  LYS  PHE  ASN  SER  SER  LEU  SER  ARG ...
T A T G A T A A A T T C A A T T C A A G C C T G A G C C G T...
          1630                 1640                 1650...
                                ... VAL  GLU  TYR  ARG  LEU  ALA  THR  HIS  GLN  SER
                                ... G T A G A A T A T C G T T T G G C A A C C C A T C A G T C T
                                ...                 1660                 1670                 1680
                                ...
```

FIG.8H

```
 TYR  GLN  LYS  LEU  ASP  TYR  THR  PRO  PRO  SER ...
T A T C A A A A A C T T G A T T A C A C C C C A C C A A G T...
          1690              1700              1710...
                        ... ASN  PRO  LEU  PRO  ASP  LYS  PHE  LYS  PRO  ILE
                        ... A A C C C T T T G C C A G A T A A G T T T A A G C C C A T T
                        ...           1720              1730              1740

 LEU  GLY  SER  ASN  ASN  LYS  PRO  ILE  CYS  LEU ...
T T A G G T T C A A A C A A C A A A C C C A T T T G C C T T...
          1750              1760              1770 ...
                        ... ASP  ALA  TYR  GLY  TYR  GLY  HIS  ASP  HIS  PRO
                        ... G A T G C T T A T G G T T A T G G T C A T G A C C A T C C A
                        ...           1780              1790              1800

 GLN  ALA  CYS  ASN  ALA  LYS  ASN  SER  THR  TYR ...
C A G G C T T G T A A C G C C A A A A A C A G C A C T T A T...
          1810              1820              1830...
                        ... GLN  ASN  PHE  ALA  ILE  LYS  LYS  GLY  ILE  GLU
                        ... C A A A A T T T T G C C A T C A A A A A A G G C A T A G A G
                        ...           1840              1850              1860

 GLN  TYR  ASN  GLN  LYS  THR  ASN  THR  ASP  LYS ...
C A A T A C A A C C A A A A A A C C A A T A C C G A T A A G...
          1870              1880              1890...
                        ... ILE  ASP  TYR  GLN  ALA  ILE  ILE  ASP  GLN  TYR
                        ... A T T G A T T A T C A A G C C A T C A T T G A C C A A T A T
                        ...           1900              1910              1920
```

FIG.8I

```
 ASP   LYS   GLN   ASN   PRO   ASN   SER   THR   LEU   LYS ...
G A T A A A C A A A A C C C C A A C A G C A C C C T A A A A...
              1930                  1940                  1950...
                                  ... PRO   PHE   GLU   LYS   ILE   LYS   GLN   SER   LEU   GLY
                                  ... C C C T T T G A G A A A A T C A A A C A A A G T T T G G G G
                                  ...                 1960                  1970                  1980

 GLN   GLU   LYS   TYR   ASN   LYS   ILE   ASP   GLU   LEU ...
C A A G A A A A A T A C A A C A A G A T A G A C G A A C T T...
              1990                  2000                  2010...
                                  ... GLY   PHE   LYS   ALA   TYR   LYS   ASP   LEU   ARG   ASN
                                  ... G G C T T T A A A G C T T A T A A A G A T T T A C G C A A C
                                  ...                 2020                  2030                  2040

 GLU   TRP   ALA   GLY   TRP   THR   ASN   ASP   ASN   SER ...
G A A T G G G C G G G T T G G A C T A A T G A C A A C A G C...
              2050                  2060                  2070...
                                  ... GLN   GLN   ASN   ALA   ASN   LYS   GLY   THR   ASP   ASN
                                  ... C A A C A A A A T G C C A A T A A A G G C A C G G A T A A T
                                  ...                 2080                  2090                  2100

 ILE   TYR   GLN   PRO   ASN   GLN   ALA   THR   VAL   VAL ...
A T C T A T C A G C C A A A T C A A G C A A C T G T G G T C...
              2110                  2120                  2130...
                                  ... LYS   ASP   ASP   LYS   CYS   LYS   TYR   SER   GLU   THR
                                  ... A A A G A T G A C A A A T G T A A A T A T A G C G A G A C C
                                  ...                 2140                  2150                  2160
```

FIG.8J

```
 ASN   SER   TYR   ALA   ASP   CYS   SER   THR   THR   ARG ...
A A C A G C T A T G C T G A T T G C T C A A C C A C T C G C...
              2170                2180                2190...
                                  ... HIS   ILE   SER   GLY   ASP   ASN   TYR   PHE   ILE   ALA
                                  ... C A C A T C A G T G G T G A T A A T T A T T T C A T C G C T
                                  ...                 2200                2210                2220

 LEU   LYS   ASP   ASN   MET   THR   ILE   ASN   LYS   TYR ...
T T A A A A G A C A A C A T G A C C A T C A A T A A A T A T...
              2230                2240                2250...
                                  ... VAL   ASP   LEU   GLY   LEU   GLY   ALA   ARG   TYR   ASP
                                  ... G T T G A T T T G G G G C T G G G T G C T C G C T A T G A C
                                  ...                 2260                2270                2280

 ARG   ILE   LYS   HIS   LYS   SER   ASP   VAL   PRO   LEU ...
A G A A T C A A A C A C A A A T C T G A T G T G C C T T T G...
              2290                2300                2310...
                                  ... VAL   ASP   ASN   SER   ALA   SER   ASN   GLN   LEU   SER
                                  ... G T A G A C A A C A G T G C C A G C A A C C A G C T G T C T
                                  ...                 2320                2330                2340

 TRP   ASN   PHE   GLY   VAL   VAL   VAL   LYS   PRO   THR ...
T G G A A T T T T G G C G T G G T C G T C A A G C C C A C C...
              2350                2360                2370...
                                  ... ASN   TRP   LEU   ASP   ILE   ALA   TYR   ARG   SER   SER
                                  ... A A T T G G C T G G A C A T C G C T T A T A G A A G C T C G
                                  ...                 2380                2390                2400
```

FIG.8K

```
 GLN  GLY  PHE  ARG  MET  PRO  SER  PHE  SER  GLU ...
C A A G G C T T T C G C A T G C C A A G T T T T T C T G A A...
          2410                 2420                 2430...
                              ... MET  TYR  GLY  GLU  ARG  PHE  GLY  VAL  THR  ILE
                              ... A T G T A T G G C G A A C G C T T T G G C G T A A C C A T C
                              ...          2440                 2450                 2460

 GLY  LYS  GLY  THR  GLN  HIS  GLY  CYS  LYS  GLY ...
G G T A A A G G C A C G C A A C A T G G C T G T A A G G G T...
          2470                 2480                 2490...
                              ... LEU  TYR  TYR  ILE  CYS  GLN  GLN  THR  VAL  HIS
                              ... C T T T A T T A C A T T T G T C A G C A G A C T G T C C A T
                              ...          2500                 2510                 2520

 GLN  THR  LYS  LEU  LYS  PRO  GLU  LYS  SER  PHE ...
C A A A C C A A G C T A A A A C C T G A A A A A T C C T T T...
          2530                 2540                 2550...
                              ... ASN  GLN  GLU  ILE  GLY  ALA  THR  LEU  HIS  ASN
                              ... A A C C A A G A A A T C G G A G C G A C T T T A C A T A A C
                              ...          2560                 2570                 2580

 HIS  LEU  GLY  SER  LEU  GLU  VAL  SER  TYR  PHE ...
C A C T T A G G C A G T C T T G A G G T T A G T T A T T T T...
          2590                 2600                 2610...
                              ... LYS  ASN  ARG  TYR  THR  ASP  LEU  ILE  VAL  GLY
                              ... A A A A A T C G C T A T A C C G A T T T G A T T G T T G G T
                              ...          2620                 2630                 2640
```

FIG.8L

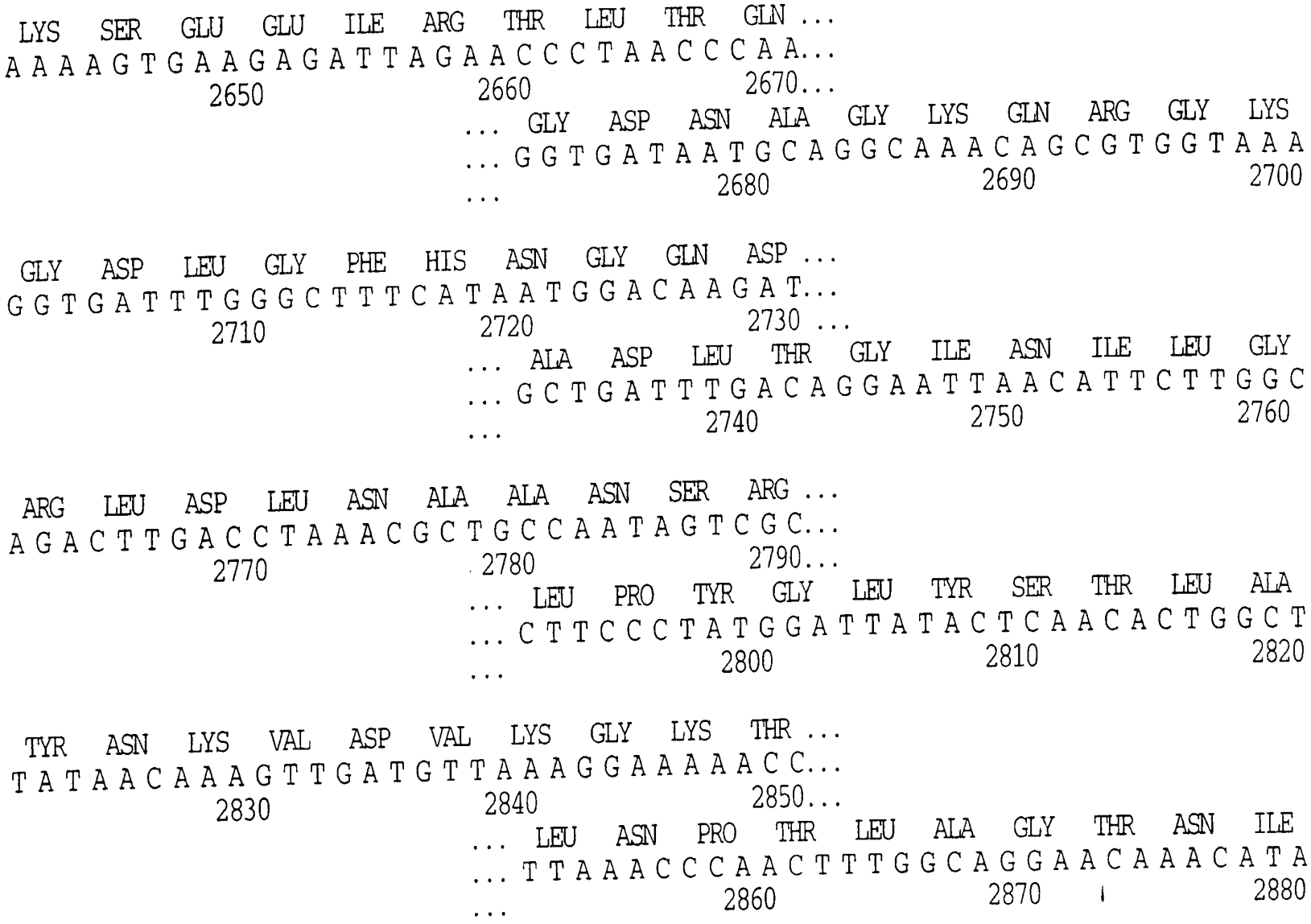


FIG.8M

```
 LEU   PHE   ASP   ALA   ILE   GLN   PRO   SER   ARG   TYR ...
C T G T T T G A T G C C A T C C A G C C A T C T C G T T A T...
              2890                2900                2910...
                                ... VAL   VAL   GLY   LEU   GLY   TYR   ASP   ALA   PRO   SER
                                ... G T G G T G G G G C T T G G C T A T G A T G C C C C A A G C
                                ...                 2920                2930                2940

 GLN   LYS   TRP   GLY   ALA   ASN   ALA   ILE   PHE   THR ...
C A A A A A T G G G G A G C A A A C G C C A T A T T T A C C...
              2950                2960                2970...
                                ... HIS   SER   ASP   ALA   LYS   ASN   PRO   SER   GLU   LEU
                                ... C A T T C T G A T G C C A A A A A T C C A A G C G A G C T T
                                ...                 2980                2990                3000

 LEU   ALA   ASP   LYS   ASN   LEU   GLY   ASN   GLY   ASN ...
T T G G C A G A T A A G A A C T T A G G T A A T G G C A A C...
              3010                3020                3030...
                                ... ILE   GLN   THR   LYS   GLN   ALA   THR   LYS   ALA   LYS
                                ... A T T C A A A C A A A A C A A G C C A C C A A A G C A A A A
                                ...                 3040                3050                3060

 SER   THR   PRO   TRP   GLN   THR   LEU   ASP   LEU   SER ...
T C C A C G C C G T G G C A A A C A C T T G A T T T G T C A...
              3070                3080                3090...
                                ... GLY   TYR   VAL   ASN   ILE   LYS   ASP   ASN   PHE   THR
                                ... G G T T A T G T A A A C A T A A A A G A T A A T T T T A C C
                                ...                 3100                3110                3120
```

FIG.8N

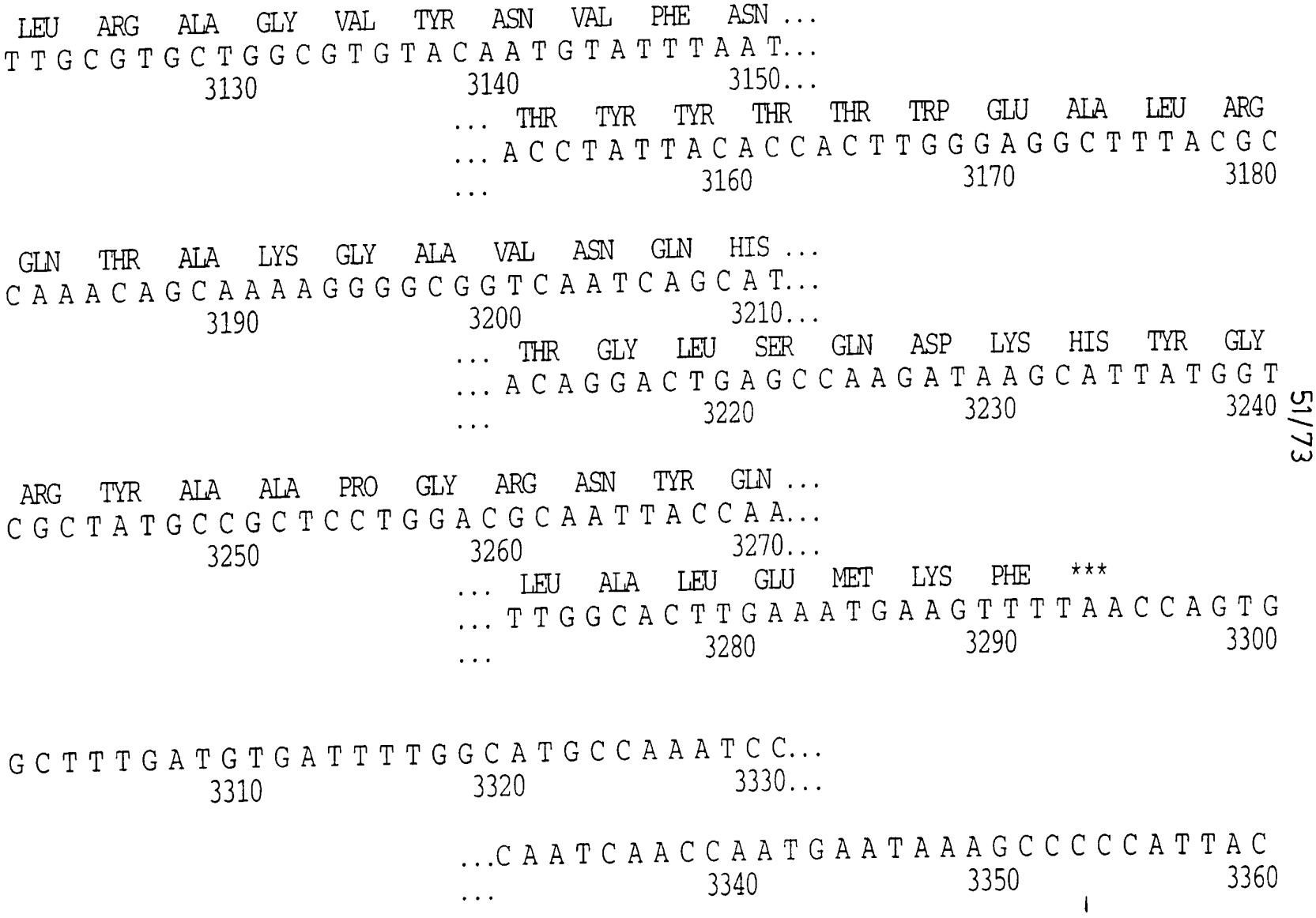

LEU ARG ALA GLY VAL TYR ASN VAL PHE ASN ...
T T G C G T G C T G G C G T G T A C A A T G T A T T T A A T...
3130 3140 3150...
... THR TYR TYR THR THR TRP GLU ALA LEU ARG
... A C C T A T T A C A C C A C T T G G G A G G C T T T A C G C
... 3160 3170 3180
GLN THR ALA LYS GLY ALA VAL ASN GLN HIS ...
C A A A C A G C A A A A G G G G C G G T C A A T C A G C A T...
3190 3200 3210...
... THR GLY LEU SER GLN ASP LYS HIS TYR GLY
... A C A G G A C T G A G C C A A G A T A A G C A T T A T G G T
... 3220 3230 3240
ARG TYR ALA ALA PRO GLY ARG ASN TYR GLN ...
C G C T A T G C C G C T C C T G G A C G C A A T T A C C A A...
3250 3260 3270...
... LEU ALA LEU GLU MET LYS PHE ***
... T T G G C A C T T G A A A T G A A G T T T T A A C C A G T G
... 3280 3290 3300
G C T T T G A T G T G A T T T T G G C A T G C C A A A T C C...
3310 3320 3330...
...C A A T C A A C C A A T G A A T A A A G C C C C C A T T A C
... 3340 3350 3360

FIG.8O

```
CATGAGGGCTTTATTTTATCATCGCTGAGT...
          3370      3380      3390...

                ...ATGCTCTTAGCGGTCATCACTCAGATTAGT
                ...       3400      3410      3420

CATTAATTTATTAGCGATTAATTTATTAGT...
          3430      3440      3450...

                ...AATCACGCTGCTCTTTGATGATTTTAAGTG
                ...       3460      3470      3480

ATGGGTATTCAAGAACGATGTCATACTCAG...
          3490      3500      3510...

                ...CACCGTTTTTATAGGCTTCTACTTCAAAGA
                ...       3520      3530      3540

CAGGCTTGCCTAAAAAGTCATCAACTTCTA...
          3550      3560      3570...

                ...TATCGCCGACTTGATAGCCACGAGCAGCAA
                ...       3580      3590      3600

GCATTTGAATGGCTTTTTGACGATTTTGGG...
          3610      3620      3630...

                ...CAAAGTTGCTGTCGCCATAAGCTTGTGCTT
                ...       3640      3650      3660
```

FIG.8P

```
TAATACGGTCGTTAGCAACTGCGGTGGTAG...
          3670          3680          3690...

                    ...AGATACCAACGGCAGGCAACAAAAACAGCAG
                    ...       3700          3710          3720

CACTTAGTACGCCAGCCAACAGTTTATTGG...
          3730          3740          3750...

                    ...TTAAATTTTTCATAGTAGTTTCCTAATTAT
                    ...       3760          3770          3780

TATCATTGTAATTCATGTTTATCGTTATAA...
          3790          3800          3810...

                    ...ACAATCGTTATAAATAACTGTGTCGTGATA
                    ...       3820          3830          3840

ACCATTAATCACAAGTGGGTTAAATGCCTT...
          3850          3860          3870...

                    ...TTGCCCAATGGCAAATAGGCACAATGCTCT
                    ...       3880          3890          3900

GCTTGTTCTATGATGGTCTATTATGATCAT...
          3910          3920          3930...

                    ...CATTTTATTGACCTATTTTTTTAATCGTAA
                    ...       3940          3950          3960
```

FIG.8Q

```
TGTTTGTTTGATGTTAGTATAAATTTTATC...
           3970          3980          3990...

                    ...AATCAAACAATCACAAATTATATCAATCAT
                    ...          4000          4010          4020

AGACGGTAAACAGGCTTCATATTTTACGCA...
           4030          4040          4050...

                    ...TATTTCCCCAGATGTCTGTAGTGTTTCATA
                    ...          4060          4070          4080

GATGATTTGTAAAACAATTGTCGGTCATTA...
           4090          4100          4110...

                    ...TTATCAATTGTAAACTGATGGCTAATTTGT
                    ...          4120          4130          4140

AACCTTATGGCTAATGATAATATGAATAAA...
           4150          4160          4170...

                    ...GCGTTATACTGTATCAAAGAATGAGTAAAA
                    ...          4180          4190          4200

ACCATCAATGGTATCTTATTTATCATCAGG...
           4210          4220          4230...

                    ...TTGTGTTAATAAGATGCCAATTAAGCGACT
                    ...          4240          4250          4260
```

FIG.8R

```
A A T T T T G T A A A T T A A T T A A T A A T C A T T C A T...
              4270                4280                4290...

                                ... A T T T G T A T T T T T A A A T A C C A T A A A A A A T G G
                                ...                 4300                4310                4320

                orf3
                MET   LEU   ALA   PHE   LEU   ILE   GLY   ALA ...
T A A A A T A T G C T C G C T T T T T T G A T A G G A G C T...
              4330                4340                4350...

                                ... VAL   MET   THR   ILE   THR   PRO   VAL   TYR   THR   THR
                                ... G T C A T G A C A A T C A C G C C T G T T T A T A C C A C A
                                ...                 4360                4370                4380

 PHE   THR   PRO   THR   LYS   THR   PRO   ILE   LYS   PHE ...
T T C A C C C C C A C C A A A A C A C C C A T A A A A T T T...
              4390                4400                4410...
                                ... PHE   MET   ALA   GLY   LEU   THR   PHE   LEU   ILE   ALA
                                ... T T T A T G G C T G G C T T G A C T T T T C T A A T C G C T
                                ...                 4420                4430                4440

 HIS   ILE   SER   HIS   ALA   ASP   ASP   GLY   ARG   THR ...
C A T A T C A G C C A T G C C G A T G A T G G T C G C A C C...
              4450                4460                4470...
                                ... ASP   ASN   GLN   GLU   LEU   ILE   ASN   GLN   GLU   ILE
                                ... G A C A A T C A A G A G C T A A T C A A T C A A G A A A T A
                                ...                 4480                4490                4500
```

FIG.8S

```
 ALA  THR  LEU  GLU  PRO  ILE  ILE  ASN  HIS  ALA ...
G C C A C C C T T G A A C C C A T C A T T A A C C A T G C T...
          4510                 4520                 4530...
                              ... GLN  PRO  GLU  LEU  LEU  SER  HIS  ASP  ALA  LEU
                              ... C A G C C T G A G T T A T T G T C C C A T G A T G C A T T A
                              ...              4540                 4550                 4560

 THR  PRO  LYS  ILE  GLU  PRO  ILE  LEU  ALA  GLN ...
A C A C C A A A A A T A G A A C C A A T A C T G G C A C A A...
          4570                 4580                 4590...
                              ... THR  PRO  ASN  PRO  ALA  GLU  ASP  THR  LEU  ILE
                              ... A C A C C A A A T C C T G C C G A A G A T A C G C T C A T C
                              ...              4600                 4610                 4620

 ALA  ASP  GLU  ALA  LEU  LEU  LEU  ASP  ASN  PRO ...
G C C G A T G A G G C G T T A C T G C T T G A T A A C C C T...
          4630                 4640                 4650...
                              ... ASP  LEU  LEU  ASN  HIS  ALA  LEU  ASN  SER  ALA
                              ... G A T T T G C T C A A T C A C G C C C T A A A T T C T G C T
                              ...              4660                 4670                 4680

 VAL  MET  THR  ASN  HIS  MET  ALA  GLY  VAL  HIS ...
G T C A T G A C C A A T C A T A T G G C A G G C G T T C A C...
          4690                 4700                 4710...
                              ... ALA  LEU  LEU  PRO  ILE  TYR  GLN  LYS  LEU  PRO
                              ... G C A T T A T T G C C C A T T T A T C A A A A A C T G C C C
                              ...              4720                 4730                 4740
```

FIG.8T

```
 LYS  ASP  HIS  GLN  ASN  GLY  ILE  LEU  LEU  GLY ...
A A A G A C C A T C A A A A T G G C A T T T T A C T T G G G...
          4750              4760              4770...
                         ... TYR  ALA  ASN  ALA  LEU  ALA  ALA  LEU  ASP  LYS
                         ... T A T G C C A A T G C C T T G G C T G C T T T G G A T A A G
                         ...               4780              4790              4800

 GLY  ASN  ALA  LYS  LYS  ALA  ILE  ASP  GLU  LEU ...
G G C A A C G C C A A A A A A G C C A T T G A T G A G C T A...
          4810              4820              4830...
                         ... ARG  ARG  ILE  ILE  ALA  ILE  MET  PRO  GLU  TYR
                         ... C G T C G C A T C A T C G C C A T C A T G C C T G A A T A T
                         ...               4840              4850              4860

 ASN  VAL  VAL  ARG  PHE  HIS  LEU  ALA  ARG  ALA ...
A A T G T G G T G C G T T T T C A T C T G G C A A G G G C A...
          4870              4880              4890...
                         ... LEU  PHE  MET  ASP  LYS  GLN  ASN  GLU  ALA  ALA
                         ... T T A T T T A T G G A C A A A C A A A A T G A A G C C G C C
                         ...               4900              4910              4920

 LEU  ASP  GLN  PHE  ASN  LYS  LEU  HIS  ALA  ASP ...
C T T G A C C A G T T T A A T A A A T T A C A T G C T G A C...
          4930              4940              4950...
                         ... ASN  LEU  PRO  GLU  GLU  VAL  ARG  GLN  VAL  VAL
                         ... A A C T T G C C A G A G G A G G T G C G G C A G G T T G T T
                         ...               4960              4970              4980
```

FIG.8U

```
 GLY  GLN  TYR  ARG  GLN  ALA  LEU  LYS  GLN  ARG ...
G G G C A G T A C A G A C A A G C G C T A A A A C A A C G A...
            4990              5000              5010...
                          ... ASP  SER  TRP  THR  TRP  GLN  VAL  GLY  MET  ASN
                          ... G A T T C A T G G A C A T G G C A A G T A G G C A T G A A T
                          ...           5020              5030              5040

 LEU  ALA  LYS  GLU  ASP  ASN  ILE  ASN  GLN  THR ...
C T G G C C A A A G A A G A C A A C A T C A A T C A A A C C...
            5050              5060              5070...
                          ... PRO  LYS  ASN  THR  THR  GLN  GLY  GLN  TRP  THR
                          ... C C C A A A A A C A C C A C G C A A G G T C A A T G G A C T
                          ...           5080              5090              5100

 PHE  ASP  LYS  PRO  ILE  ASP  ALA  ILE  THR  LEU ...
T T T G A C A A A C C C A T T G A C G C C A T C A C C C T A...
            5110              5120              5130...
                          ... SER  TYR  GLN  LEU  GLY  ALA  ASP  LYS  LYS  TRP
                          ... A G C T A C C A A T T G G G G G C G G A T A A A A A G T G G
                          ...           5140              5150              5160

 SER  LEU  PRO  LYS  GLY  ALA  TYR  VAL  GLY  ALA ...
T C T T T G C C C A A A G G G G C A T A T G T G G G A G C G...
            5170              5180              5190...
                          ... ASN  ALA  GLN  ILE  TYR  GLY  LYS  HIS  HIS  GLN
                          ... A A C G C C C A A A T C T A T G G C A A A C A T C A T C A A
                          ...           5200              5210              5220
```

FIG.8V

```
 ASN  HIS  LYS  LYS  TYR  ASN  ASP  HIS  TRP  GLY ...
A A T C A C A A A A A A T A C A A C G A C C A T T G G G G C...
            5230                 5240                 5250...
                                ... ARG  LEU  GLY  ALA  ASN  LEU  GLY  PHE  ALA  ASP
                                ... A G A C T G G G G G C A A A T T T G G G C T T T G C T G A T
                                ...                 5260                 5270                 5280

 ALA  LYS  LYS  ASP  LEU  SER  ILE  GLU  THR  TYR ...
G C C A A A A A A G A C C T T A G C A T T G A G A C C T A T...
            5290                 5300                 5310...
                                ... GLY  GLU  LYS  ARG  PHE  TYR  GLY  HIS  GLU  ARG
                                ... G G T G A A A A A A G A T T T T A T G G G C A T G A G C G T
                                ...                 5320                 5330                 5340

 TYR  THR  ASP  THR  ILE  GLY  ILE  ARG  MET  SER ...
T A T A C C G A C A C C A T T G G C A T A C G C A T G T C G...
            5350                 5360                 5370...
                                ... VAL  ASP  TYR  ARG  ILE  ASN  PRO  LYS  PHE  GLN
                                ... G T T G A T T A T A G A A T C A A C C C A A A A T T T C A A
                                ...                 5380                 5390                 5400

 SER  LEU  ASN  ALA  ILE  ASP  ILE  SER  ARG  LEU ...
A G C C T A A A C G C C A T A G A C A T A T C A C G C C T A...
            5410                 5420                 5430...
                                ... THR  ASN  HIS  ARG  THR  PRO  ARG  ALA  ASP  SER
                                ... A C C A A C C A T C G G A C G C C T A G G G C T G A C A G T
                                ...                 5440                 5450                 5460
```

FIG.8W

```
 ASN  ASN  THR  LEU  TYR  SER  THR  SER  LEU  ILE ...
AATAACACTTTATACAGTACCTCATTGATT...
          5470           5480           5490...
                    ... TYR  TYR  PRO  ASN  ALA  THR  ARG  TYR  TYR  LEU
                    ...TATTACCCAAATGCCACACGCTATTATCTT
                    ...          5500           5510           5520

 LEU  GLY  ALA  ASP  PHE  TYR  ASP  GLU  LYS  VAL ...
TTGGGGGCAGACTTTTATGATGAAAAAGTG...
          5530           5540           5550...
                    ... PRO  GLN  ASP  PRO  SER  ASP  SER  TYR  GLN  ARG
                    ...CCACAAGACCCATCTGACAGTTATCAACGC
                    ...          5560           5570           5580

 ARG  GLY  ILE  ARG  THR  ALA  TRP  GLY  GLN  GLU ...
CGTGGCATACGCACAGCGTGGGGGCAAGAA...
          5590           5600           5610...
                    ... TRP  ALA  GLY  GLY  LEU  SER  SER  ARG  ALA  GLN
                    ...TGGGCGGGTGGTCTTTCAAGCCGTGCCCAA
                    ...          5620           5630           5640

 ILE  SER  ILE  ASN  LYS  ARG  HIS  TYR  GLN  GLY ...
ATCAGCATCAACAAACGCCATTACCAAGGG...
          5650           5660           5670...
                    ... ALA  ASN  LEU  THR  SER  GLY  GLY  GLN  ILE  ARG
                    ...GCAAACCTAACCAGCGGTGGACAAATTCGC
                    ...          5680           5690           5700
```

FIG.8X

```
 HIS  ASP  LYS  GLN  MET  GLN  ALA  SER  LEU  SER ...
C A T G A T A A A C A G A T G C A A G C G T C T T T A T C G...
            5710                 5720                 5730...
                                ... LEU  TRP  HIS  ARG  ASP  ILE  HIS  LYS  TRP  GLY
                                ... C T T T G G C A C A G A G A C A T T C A C A A A T G G G G C
                                ...                 5740                 5750                 5760

 ILE  THR  PRO  ARG  LEU  THR  ILE  SER  THR  ASN ...
A T C A C G C C A C G G C T G A C C A T C A G C A C A A A C...
            5770                 5780                 5790...
                                ... ILE  ASN  LYS  SER  ASN  ASP  ILE  LYS  ALA  ASN
                                ... A T C A A T A A A A G C A A T G A C A T C A A G G C A A A T
                                ...                 5800                 5810                 5820

 TYR  HIS  LYS  ASN  GLN  MET  PHE  VAL  GLU  PHE ...
T A T C A C A A A A A T C A A A T G T T T G T T G A G T T T...
            5830                 5840                 5850...
                                ... SER  ARG  ILE  PHE  ***
                                ... A G T C G C A T T T T T T G A T G G G A T A A G C A C G C C
                                ...                 5860                 5870                 5880


C T A C T T T T G T T T T T G T A A A A A A A T G T G C C A...
            5890                 5900                 5910...

                                ... T C A T A G A C A A T A T C A A G A A A A A A T C A A G A A
                                ...                 5920                 5930                 5940
```

FIG.8Y

```
A A A A A G A T T A C A A A T T T A A T G A T A A T T G T T...
            5950              5960              5970...

                        ... A T T G T T T A T G T T A T T A T T T A T C A A T G T A A A
                        ...             5980              5990              6000

T T T G C C G T A T T T T G T C T A T C A T A A A T G C A T...
            6010              6020              6030...

                        ... T T A T C A A A T G C T C A A A T A A A T A C G C C A A A T
                        ...             6040              6050              6060

G C A C A T T G T C A G C A T G C C A A A A T A G G C A T C...
            6070              6080              6090...

                        ... A A C A G A C T T T T T T A G A T A A T A C C A T C A A C C
                        ...             6100              6110              6120

                          tbpB
                          MET   LYS   HIS   ILE   ...
C A T C A G A G G A T T A T T T T A T G A A A C A C A T T C...
            6130              6140              6150...

                        ...PRO   LEU   THR   THR   LEU   CYS   VAL   ALA   ILE   SER   A
                        ... C T T T A A C C A C A C T G T G T G T G G C A A T C T C T G
                        ...             6160              6170              6180
```

FIG.8Z

```
LA  VAL  LEU  LEU  THR  ALA  CYS  GLY  GLY  SER  ...
C C G T C T T A T T A A C C G C T T G T G G T G G C A G T G...
            6190              6200              6210...
                                ...GLY  GLY  SER  ASN  PRO  PRO  ALA  PRO  THR  PRO  I
                                ... G T G G T T C A A A T C C A C C T G C T C C T A C G C C C A
                                ...              6220              6230              6240

LE  PRO  ASN  ALA  SER  GLY  SER  GLY  ASN  THR  ...
T T C C A A A T G C T A G C G G T T C A G G T A A T A C T G...
            6250              6260              6270...
                                ...GLY  ASN  THR  GLY  ASN  ALA  GLY  GLY  THR  ASP  A
                                ... G C A A C A C T G G T A A T G C T G G C G G T A C T G A T A
                                ...              6280              6290              6300

SN  THR  ALA  ASN  ALA  GLY  ASN  THR  GLY  GLY  ...
A T A C A G C C A A T G C A G G T A A T A C A G G C G G T A...
            6310              6320              6330...
                                ...THR  ASN  SER  GLY  THR  GLY  SER  ALA  ASN  THR  P
                                ... C A A A C T C T G G T A C A G G C A G T G C C A A C A C A C
                                ...              6340              6350              6360

RO  GLU  PRO  LYS  TYR  GLN  ASP  VAL  PRO  THR  ...
C A G A G C C A A A A T A T C A A G A T G T A C C A A C T G...
            6370              6380              6390...
                                ...GLU  LYS  ASN  GLU  LYS  ASP  LYS  VAL  SER  SER  I
                                ... A G A A A A A T G A A A A A G A T A A A G T T T C A T C C A
                                ...              6400              6410              6420
```

FIG.8A'

```
LE  GLN  GLU  PRO  ALA  MET  GLY  TYR  GLY  MET  ...
T T C A A G A A C C T G C C A T G G G T T A T G G C A T G G...
             6430              6440              6450...
                               ...ALA  LEU  SER  LYS  ILE  ASN  LEU  HIS  ASN  ARG  G
                               ... C T T T G A G T A A A A T T A A T C T A C A C A A C C G A C
                               ...              6460              6470              6480

LN  ASP  THR  PRO  LEU  ASP  GLU  LYS  ASN  ILE  ...
A A G A C A C G C C A T T A G A T G A A A A A A A T A T C A...
             6490              6500              6510...
                               ...ILE  THR  LEU  ASP  GLY  LYS  LYS  GLN  VAL  ALA  G
                               ... T T A C C T T A G A C G G T A A A A A A C A A G T T G C A G
                               ...              6520              6530              6540

LU  GLY  LYS  LYS  SER  PRO  LEU  PRO  PHE  SER  ...
A A G G T A A A A A A T C G C C A T T G C C A T T T T C G T...
             6550              6560              6570...
                               ...LEU  ASP  VAL  GLU  ASN  LYS  LEU  LEU  ASP  GLY  T
                               ... T A G A T G T A G A A A A T A A A T T G C T T G A T G G C T
                               ...              6580              6590              6600

YR  ILE  ALA  LYS  MET  ASN  VAL  ALA  ASP  LYS  ...
A T A T A G C A A A A A T G A A T G T A G C G G A T A A A A...
             6610              6620              6630...
                               ...ASN  ALA  ILE  GLY  ASP  ARG  ILE  LYS  LYS  GLY  A
                               ... A T G C C A T T G G T G A C A G A A T T A A G A A A G G T A
                               ...              6640              6650              6660
```

FIG.8B'

```
SN  LYS  GLU  ILE  SER  ASP  GLU  GLU  LEU  ALA  ...
A T A A A G A A A T C T C C G A T G A A G A A C T T G C C A...
          6670              6680              6690...
                           ...LYS  GLN  ILE  LYS  GLU  ALA  VAL  ARG  LYS  SER  H
                           ... A A C A A A T C A A A G A A G C T G T G C G T A A A A G C C
                           ...            6700              6710              6720

IS  GLU  PHE  GLN  GLN  VAL  LEU  SER  SER  LEU  ...
A T G A G T T T C A G C A A G T A T T A T C A T C A C T G G...
          6730              6740              6750...
                           ...GLU  ASN  LYS  ILE  PHE  HIS  SER  ASN  ASP  GLY  T
                           ... A A A A C A A A A T T T T T C A T T C A A A T G A C G G A A
                           ...            6760              6770              6780

HR  THR  LYS  ALA  THR  THR  ARG  ASP  LEU  LYS  ...
C A A C C A A A G C A A C C A C A C G A G A T T T A A A A T...
          6790              6800              6810...
                           ...TYR  VAL  ASP  TYR  GLY  TYR  TYR  LEU  ALA  ASN  A
                           ... A T G T T G A T T A T G G T T A C T A C T T G G C G A A T G
                           ...            6820              6830              6840

SP  GLY  ASN  TYR  LEU  THR  VAL  LYS  THR  ASP  ...
A T G G C A A T T A T C T A A C C G T C A A A A C A G A C A...
          6850              6860              6870...
                           ...LYS  LEU  TRP  ASN  LEU  GLY  PRO  VAL  GLY  GLY  V
                           ... A A C T T T G G A A T T T A G G C C C T G T G G G T G G T G
                           ...            6880              6890              6900
```

FIG.8C'

```
AL  PHE  TYR  ASN  GLY  THR  THR  THR  ALA  LYS  ...
TGTTTTATAATGGCACAACGACCGCCAAAG...
          6910           6920           6930...
                        ...GLU  LEU  PRO  THR  GLN  ASP  ALA  VAL  LYS  TYR  L
                        ... AGTTGCCCACACAAGATGCGGTCAAATATA
                        ...          6940           6950           6960

YS  GLY  HIS  TRP  ASP  PHE  MET  THR  ASP  VAL  ...
AAGGACATTGGGACTTTATGACCGATGTTG...
          6970           6980           6990...
                        ...ALA  ASN  ARG  ARG  ASN  ARG  PHE  SER  GLU  VAL  L
                        ... CCAACAGAAGAAACCGATTTAGCGAAGTGA
                        ...          7000           7010           7020

YS  GLU  ASN  SER  GLN  ALA  GLY  TRP  TYR  TYR  ...
AAGAAAACTCTCAAGCAGGCTGGTATTATG...
          7030           7040           7050...
                        ...GLY  ALA  SER  SER  LYS  ASP  GLU  TYR  ASN  ARG  L
                        ... GAGCATCTTCAAAAGATGAATACAACCGCT
                        ...          7060           7070           7080

EU  LEU  THR  LYS  GLU  ASP  SER  ALA  PRO  ASP  ...
TATTAACTAAAGAAGACTCTGCCCCTGATG...
          7090           7100           7110...
                        ...GLY  HIS  SER  GLY  GLU  TYR  GLY  HIS  SER  SER  G
                        ... GTCATAGCGGTGAATATGGCCATAGCAGTG
                        ...          7120           7130           7140
```

FIG.8D'

```
LU  PHE  THR  VAL  ASN  PHE  LYS  GLU  LYS  LYS  ...
AGTTTACTGTTAATTTTAAGGAAAAAAAAT...
          7150           7160           7170...
                        ...LEU  THR  GLY  LYS  LEU  PHE  SER  ASN  LEU  GLN  A
                        ... TAACAGGTAAGCTGTTTAGTAACCTACAAG
                        ...          7180           7190           7200

SP  ARG  HIS  LYS  GLY  ASN  VAL  THR  LYS  THR  ...
ACCGCCATAAGGGCAATGTTACAAAAACCG...
          7210           7220           7230...
                        ...GLU  ARG  TYR  ASP  ILE  ASP  ALA  ASN  ILE  HIS  G
                        ... AACGCTATGACATCGATGCCAATATCCACG
                        ...          7240           7250           7260

LY  ASN  ARG  PHE  ARG  GLY  SER  ALA  THR  ALA  ...
GCAACCGCTTCCGTGGCAGTGCCACCGCAA...
          7270           7280           7290...
                        ...SER  ASN  LYS  ASN  ASP  THR  SER  LYS  HIS  PRO  P
                        ... GCAATAAAAATGACACAAGCAAACACCCCT
                        ...          7300           7310           7320

HE  THR  SER  ASP  ALA  ASN  ASN  ARG  LEU  GLU  ...
TTACCAGTGATGCCAACAATAGGCTAGAAG...
          7330           7340           7350...
                        ...GLY  GLY  PHE  TYR  GLY  PRO  LYS  GLY  GLU  GLU  L
                        ... GTGGTTTTTATGGGCCAAAAGGCGAGGAGC
                        ...          7360           7370           7380
```

FIG.8E'

```
EU  ALA  GLY  LYS  PHE  LEU  THR  ASN  ASP  ASN  ...
T G G C A G G T A A A T T C T T A A C C A A T G A C A A C A...
            7390              7400              7410...
                              ...LYS  LEU  PHE  GLY  VAL  PHE  GLY  ALA  LYS  ARG  G
                              ... A A C T C T T T G G C G T C T T T G G T G C T A A A C G A G
                              ...            7420              7430              7440

LU  SER  LYS  ALA  GLU  GLU  LYS  THR  GLU  ALA  ...
A G A G T A A A G C T G A G G A A A A A A C C G A A G C C A...
            7450              7460              7470...
                              ...ILE  LEU  ASP  ALA  TYR  ALA  LEU  GLY  THR  PHE  A
                              ... T C T T A G A T G C C T A T G C A C T T G G G A C A T T T A
                              ...            7480              7490              7500

SN  THR  SER  ASN  ALA  THR  THR  PHE  THR  PRO  ...
A T A C A A G T A A C G C A A C C A C A T T C A C C C C A T...
            7510              7520              7530...
                              ...PHE  THR  GLU  LYS  GLN  LEU  ASP  ASN  PHE  GLY  A
                              ... T T A C C G A A A A A C A A C T G G A T A A C T T T G G C A
                              ...            7540              7550              7560

SN  ALA  LYS  LYS  LEU  VAL  LEU  GLY  SER  THR  ...
A T G C C A A A A A A T T G G T C T T A G G T T C T A C C G...
            7570              7580              7590...
                              ...VAL  ILE  ASP  LEU  VAL  PRO  THR  ASP  ALA  THR  L
                              ... T C A T T G A T T T G G T G C C T A C T G A T G C C A C C A
                              ...            7600              7610              7620
```

FIG.8F'

```
YS  ASN  GLU  PHE  THR  LYS  ASP  LYS  PRO  GLU  ...
A A A A T G A A T T C A C C A A A G A C A A G C C A G A G T...
          7630              7640              7650...
                           ...SER  ALA  THR  ASN  GLU  ALA  GLY  GLU  THR  LEU  M
                           ... C T G C C A C A A A C G A A G C G G G C G A G A C T T T G A
                           ...            7660              7670              7680

ET  VAL  ASN  ASP  GLU  VAL  SER  VAL  LYS  THR  ...
T G G T G A A T G A T G A A G T T A G C G T C A A A A C C T...
          7690              7700              7710...
                           ...TYR  GLY  LYS  ASN  PHE  GLU  TYR  LEU  LYS  PHE  G
                           ... A T G G C A A A A A C T T T G A A T A C C T A A A A T T T G
                           ...            7720              7730              7740

LY  GLU  LEU  SER  ILE  GLY  GLY  SER  HIS  SER  ...
G T G A G C T T A G T A T C G G T G G T A G C C A T A G C G...
          7750              7760              7770...
                           ...VAL  PHE  LEU  GLN  GLY  GLU  ARG  THR  ALA  THR  T
                           ... T C T T T T T A C A A G G C G A A C G C A C C G C T A C C A
                           ...            7780              7790              7800

HR  GLY  GLU  LYS  ALA  VAL  PRO  THR  THR  GLY  ...
C A G G C G A G A A A G C C G T A C C A A C C A C A G G C A...
          7810              7820              7830...
                           ...THR  ALA  LYS  TYR  LEU  GLY  ASN  TRP  VAL  GLY  T
                           ... C A G C C A A A T A T T T G G G G A A C T G G G T A G G A T
                           ...            7840              7850              7860
```

FIG.8G'

```
YR  ILE  THR  GLY  LYS  ASP  THR  GLY  THR  GLY  ...
A C A T C A C A G G A A A G G A C A C A G G A A C G G G C A...
              7870                 7880                 7890...
                                  ...THR  GLY  LYS  SER  PHE  THR  ASP  ALA  GLN  ASP  V
                                  ... C A G G A A A A A G C T T T A C C G A T G C C C A A G A T G
                                  ...              7900                 7910                 7920

AL  ALA  ASP  PHE  ASP  ILE  ASP  PHE  GLY  ASN  ...
T T G C T G A T T T T G A C A T T G A T T T T G G A A A T A...
              7930                 7940                 7950...
                                  ...LYS  SER  VAL  SER  GLY  LYS  LEU  ILE  THR  LYS  G
                                  ... A A T C A G T C A G C G G T A A A C T T A T C A C C A A A G
                                  ...              7960                 7970                 7980

LY  ARG  GLN  ASP  PRO  VAL  PHE  SER  ILE  THR  ...
G C C G C C A A G A C C C T G T A T T T A G C A T C A C A G...
              7990                 8000                 8010...
                                  ...GLY  GLN  ILE  ALA  GLY  ASN  GLY  TRP  THR  GLY  T
                                  ... G T C A A A T C G C A G G C A A T G G C T G G A C A G G G A
                                  ...              8020                 8030                 8040

HR  ALA  SER  THR  THR  LYS  ALA  ASP  ALA  GLY  ...
C A G C C A G C A C C A C C A A A G C G G A C G C A G G A G...
              8050                 8060                 8070...
                                  ...GLY  TYR  LYS  ILE  ASP  SER  SER  SER  THR  GLY  L
                                  ... G C T A C A A G A T A G A T T C T A G C A G T A C A G G C A
                                  ...              8080                 8090                 8100
```

FIG.8H'

```
YS   SER   ILE   ALA   ILE   LYS   ASP   ALA   ASN   VAL   ...
AATCCATCGCCATCAAAGATGCCAATGTTA...
            8110              8120              8130...
                             ...THR   GLY   GLY   PHE   TYR   GLY   PRO   ASN   ALA   ASN   G
                             ... CAGGGGGCTTTTATGGTCCAAATGCAAACG
                             ...           8140              8150              8160

LU   MET   GLY   GLY   SER   PHE   THR   HIS   ASN   ALA   ...
AGATGGGCGGGTCATTTACACACAACGCCG...
            8170              8180              8190...
                             ...ASP   ASP   SER   LYS   ALA   SER   VAL   VAL   PHE   GLY   T
                             ... ATGACAGCAAAGCCTCTGTGGTCTTTGGCA
                             ...           8200              8210              8220

HR   LYS   ARG   GLN   GLN   GLU   VAL   LYS   ***
CAAAAAGACAACAAGAAGTTAAGTAGTAAT...
            8230              8240              8250...

                             ... TTAAACACAATGTTTG
                             ...           8260
```

FIG.9A

Alignment of *M. catarrhalis* ORF3 proteins

```
        10         20         30         40         50
MLAFLIGAVMTITPVYTTFTPTKTPIKFFMAGLTFLIAHISHADDGRTDN
..................................................
                                          60         70         80         90         100
                                  ..P.....................G.....T.................  Q8
                                  QELINQEIATLEPIINHAQPELLSHDALTPKIEPILAQTPNPAEDTLIAD  4223

       110        120        130        140        150
EALLLDNPDLLNHALNSAVMTNHMAGVHALLPIYQKLPKDHQNGILLGYA
.....................N........................
                                            160        170        180        190        200
                                   NALAALDKGNAKKAIDELRRIIAIMPEYNVVRFHLARALFMDKQNEAALD  4223
                                   ...V.........A..G.................................  Q8

       210        220        230        240        250
QFNKLHADNLPEEVRQVVGQYRQALKQRDSWTWQVGMNLAKEDNINQTPK
..............R...................................
                                            260        270        280        290        300
                                   NTTQGQWTFDKPIDAITLSYQLGADKKWSLPKGAYVGANAQIYGKHHQNH  4223
                                   ..................................................  Q8

       310        320        330        340        350
KKYNDHWGRLGANLGFADAKKDLSIETYGEKRFYGHERYTDTIGIRMSVD
..................................................A.
                                            360        370        380        390        400
                                   YRINPKFQSLNAIDISRLTNHRTPRADSNNTLYSTSLIYYPNATRYYLLG  4223
                                   ..................................................  Q8
```

FIG.9B

```
          410       420       430       440       450
ADFYDEKVPQDPSDSYQRRGIRTAWGQEWAGGLSSRAQISINKRHYQGAN
.................E................................
                                                  460       470       480       490       500
                                                  LTSGGQIRHDKQMQASLSLWHRDIHKWGITPRLTISTNINKSNDIKANYH  4223
                                                  ........Q.........................................  Q8

          510
KNQMFVEFSRIF*
............*
```

4223
Q8

INTERNATIONAL SEARCH REPORT

Int Application No
PCT/CA 99/00307

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07K14/79 C07K14/22 C12N15/31 C12Q1/68 A61K39/02
A61K48/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97 32980 A (CONNAUGHT LAB) 12 September 1997 (1997-09-12) page 3, line 29 -page 9, line 32 page 19, line 32 -page 30, line 6; examples 1-19 SEQ.ID.N.3 ---	1-12
A	WO 97 13785 A (CONNAUGHT LAB ;YANG YAN PING (CA); MYERS LISA E (CA); HARKNESS ROB) 17 April 1997 (1997-04-17) page 1, line 1 -page 3, line 8; examples 1-8 page 4, line 20 -page 8, line 31 ---	1,9
A	US 5 708 149 A (SCHRYVERS ANTHONY ET AL) 13 January 1998 (1998-01-13) abstract; figure 23 column 5, line 63 -column 6, line 28 ---	1,6
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 October 1999	02/11/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Mateo Rosell, A.M.

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	SCHRYVERS A B ET AL: "COMPARATIVE ANALYSIS OF THE TRANSFERRIN AND LACTOFERRIN BINDING PROTEINS IN THE FAMILY NEISSERIACEAE" CANADIAN JOURNAL OF MICROBIOLOGY, vol. 35, no. 5, 1 May 1989 (1989-05-01), pages 409-415, XP002020995 ISSN: 0008-4166 cited in the application abstract	1
A	RAONG-HUA YU ET AL: "THE INTERACTION BETWEEN HUMAN TRANSFERRIN AND TRANSFERRIN BINDING PROTEIN 2 FROM MORAXELLA (BRANHAMELLA) CATARRHALIS DIFFERS FROM THAT OF OTHER HUMAN PATHOGENS" MICROBIAL PATHOGENESIS, vol. 15, 1 January 1993 (1993-01-01), pages 433-445, XP000612196 ISSN: 0882-4010 abstract	1
P,X	MYERS L.E. ET AL., : "The transferrin binding protein B of moraxella catarrhalis elicits bactericidal antibodies and is a potential vaccine antigen" INFECTION AND IMMUNITY, vol. 66, no. 9, 1998, page 4183-4192 XP002118475 the whole document	2,7

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA 99/00307

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 9
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claim 9 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/CA 99/00307

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9732980 A	12-09-1997	AU 1865397 A	22-09-1997
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